UNIVERSITY OF ALBERTA

RESPONSE OF NEAR-ISOGENIC WHEAT LINES FOR AN ALUMINUM
TOLERANCE GENE TO VARIABLE LEVELS OF ALUMINUM STRESS IN A
SOIL SYSTEM

by

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Dedication

To my parents, brother and friends

ABSTRACT

Near-isogenic lines for aluminum tolerance, Alikat (tolerant) and Katepwa (sensitive) were studied in the greenhouse at a soil pH 4.4-7.0 in acid gleysol from northern Alberta to determine the range of effectiveness of a dominant aluminum tolerance gene from the Brazilian cultivar Maringa under a soil-pH range. Previous characterization had been carried out in hydroponic systems. Root, shoot and total weight of Katepwa was half of Alikat at pH 4.4-5.5 but were similar above pH 5.5, consistent with solution culture responses. Alikat seed yield was four times that of Katepwa at pH 4.4, showing the advantage of Al-tolerance under extreme stress. Soil pH, genotype tolerance, and growth period affected Haun-scale values, length and width of the first leaf, leaf area development, and root and shoot weight increase. In conclusion, root and leaf area development are improved by the aluminum tolerance gene in wheat when grown in an aluminum-toxic soil .

ACKNOWLEDGMENTS

Contents   Page

4. Leaf growth during the early seedling stage in near-isogenic genotypes for aluminum tolerance under variable soil pH having varying levels of aluminum toxicity

List of figures   Page

Chapter I

Introduction and literature review

1.1 General introduction

Spring wheat is an important crop in Canada occupying 6.85 million acres (seeded) out of a total 7.745 million acres of all wheat seeded (Statistics Canada, 1996) and can be grown in soils that are neutral to slightly alkaline in pH. In areas where the soil is acidic and the pH is below 5.0, the wheat crop primarily suffers from aluminum toxicity (Foy, 1988). Toxic levels of aluminum available to plants in acid soils results in decrease in crop yield (Foy, 1988). The solution to this problem lies in the use of agronomic practices such as liming of soil in conjunction with breeding crop varieties which are tolerant to aluminum (Foy, 1988). Aluminum tolerance is thought to be due to a single major dominant gene (Minella and Sorrells, 1992; Somers et al. 1996; Somers and Gustafson, 1996; Taylor et al. 1996) and some modifier genes (Berzhonsky, 1992; Camargo, 1981, 1984; Campbell and LaFever, 1981). Nyachiro (1986), and Zale (1988) showed that the inheritance of aluminum tolerance followed simple Mendelian patterns. The growth patterns of near-isogenic lines of spring wheat for aluminum tolerance in aluminum toxic media indicate basic differences caused by aluminum tolerance gene(s), and some understanding of the mechanism(s) of tolerance and growth under toxicity has been gained from the use of these isogenics. There are several methods of selecting for aluminum tolerance (Little, 1988). Hydroponic studies have usually been done using high aluminum levels and low pH values rather than a range of stress levels. The nutrient culture medium does not exactly represent or simulate the actual field condition even though it offers a better control over the toxicity factor and level (Little, 1988). Use of soil medium in a greenhouse experiment can also be used as an experimental approach. In soils having toxic levels of aluminum, drought stress has been said to be an additional stress factor (Krizek et al. 1988; Goldman et al. 1989). Effects of drought stress usually appear on leaf growth during the early stage of plant development (Boyer, 1985; Levitt, 1972; Rosenthal et al. 1987). The vegetative growth of wheat has been described using the Haun scale (LaFond and Baker, 1986) and for quantification of early growth this method has been said to be the most sensitive to environmental stress factors (Bauer et al. 1984). An effort has been made in this thesis to study all the above aspects of aluminum toxicity in wheat plants. Use of the near-isogenic approach (to offer a high level of genetic control) in combination with a range of soil pH to test response has not been reported in the literature, and is of

interest to characterize the effect(s) of single genes in aluminum tolerance(s), especially in the earliest stages of growth.

1.2 Literature review

1. 2.1 World wheat production

World wheat (*Triticum aestivum* L.) production in 1994 was estimated at 528 million metric tons, a 6% decrease from the previous year's 564 million tons (FAO, 1995). The rate of growth in wheat production in the world has increased from 1955 to 1984 but has decreased dramatically in the last decade (Rejesus 1995). In 1955-64 it was 1.7%, it increased to 3.4% in 1975-84 but decreased to 1.5% in 1985-1994. Increase in wheat production has mainly been attributable to better yielding semidwarf varieties and improvement in agricultural inputs. Thus the wheat yields increased at a steady rate from the 1960's to the 1970's but since then the increase has slowed. The reasons were decrease in wheat cultivation areas, low domestic prices, uncertainty regarding weather conditions and crop diversification in certain areas. Problems such as soil acidification have further contributed to reducing wheat yield (Fageria and Baligar, 1993), although bread wheat (*Triticum aestivum* L.) is relatively less sensitive to acid soil than highly sensitive crops such as barley (*Hordeum vulgare* L.) and durum wheat (*Triticum durum* L.). When soil pH (1:1 w/w ratio of soil and water) decreases below 5.0 to 5.2 forage and grain yields are reduced (Westerman 1987). Therefore the minimum soil pH recommended for wheat cultivation is 5.5. It is a world wide occurrence that wheat is cultivated in acid soil areas where the pH falls short of the recommended level either in the top soil or subsoil or both. Prevention or correction of soil acidity and crop tolerance to acid soil conditions are the solutions which are prescribed for wheat cultivation in such problem soils.

1.2.2 Quantification of wheat growth and development

Vegetative growth in wheat has been studied with the use of the phyllocron concept (Wilhelm and McMaster, 1995). This concept is useful in the development of crop simulation models to predict crop growth in several crops including spring wheat (Miglietta, 1991; Cutforth et al. 1992) and winter wheat (Cao and Moss, 1991; Krenzer et al. 1991). The successive appearance of leaves on the stem and tillers has been described using developmental scales such as the Zadoks' scale (Zadoks et al. 1971), Feekes' scale (1941) and Haun scale (Haun, 1973). Growth can be defined as an increase in volume (Salisbury and Ross, 1969; Sinnott, 1960; and Wetmore and Steeves, 1971). The increase in leaf area and increase in the leaf blade length are signs of growth. In agricultural terms, growth can be

measured by an increase in the total dry weight of the plant. Several environmental factors seem to affect the phyllochron (Wilhelm and McMaster, 1995) including water stress (Baker et al. 1986; Bauer et al. 1984) and nutrient availability (Bauer et al. 1984; Frank and Bauer, 1982). Haun Scale is used to determine the phyllochron of a plant during its vegetative growth by using the following formula for the Haun Scale (Haun, 1973).

$$\text{Haun Stage} = [L_n / L_{(n-1)}] + (n-1)$$

where L_n denotes the length of the youngest leaf above the collar of the previous leaf, $L_{(n-1)}$ denotes the length of the penultimate leaf and n is the total number of leaves on the culm. The phyllochron can be further utilized in understanding and describing the development of grasses and the interaction effects between the genotype and the environment (Rickman and Klepper, 1995). The Haun scale can be used to describe the rate of development in early growth of the wheat seedling, in terms of rate of leaf emergence, independent of the assessment of plant leaf area development.

1.2.3 The problem

1.2.3.1 Soil acidity

Soil acidity is a serious problem throughout the world (Van Wambeke, 1976) and it occurs in as much as 40% of the world's total arable soils (Haug, 1984). Acid soils have long been known to cause problems for the cultivation of several crop species. Recently a great deal of attention has been drawn to the problem of soil acidification and its effect on wheat (*Triticum aestivum* L.) production. Soil acidity was once considered as a specific problem for tropical regions but nowadays it is a global problem attracting the attention of wheat producers in the United States, Canada, Australia, South America, Carpathian region of Europe, Central Africa and South Africa.

In Canada, surface and subsurface soil acidity is observed in the provinces of Alberta and British Columbia (Penney et al. 1977). In the Peace River region in Alberta and British Columbia subsoil acidity has been observed (Penney et al. 1977). In a review by Briggs and Taylor (1994) it was suggested that approximately 5.5 million hectares of cultivable soil in Canada are acidic with 2.0 million hectares in western Canada alone where wheat is an important crop. Soil acidity is measured by the amount of H^+ activity in soil solution and is influenced by edaphic, climatic, and biological factors (Johnson, 1986). Soils having a granitic origin develop acidity faster than those having a calcareous origin. Sandy soils have low cation exchange capacity and a high leaching potential and acidify quickly due to the shortage of alkaline cations. Rainfall increases percolation of water through the soil profile

and when the rainwater is acidic it further increases acidification. Organic matter decays and forms acids, such as carbonic and other weak organic acids. Soil acidity is also enhanced due to certain agronomic practices such as repeated applications of nitrogenous fertilizers on the top soil. Removal of straw from the field depletes the soil cations and enhances acidification by nitrification (Westerman, 1987). In Brazil the condition known as 'crestamento' which in Portuguese means 'burning' or 'toasting' has been known as early as 1925 (da Silva, 1976). Acid soils have high levels of exchangeable metal ions such as aluminum, iron and manganese and toxicity of hydrogen ions (H^+). On the other hand they are also generally deficient in calcium, magnesium, phosphorus and molybdenum. The toxicities produced in certain plant species are not a function of soil pH (Mugwira et al. 1981) rather they are most often due to aluminum toxicity (Araujo, 1948, 1949). It has been estimated that approximately 55% of the soils in tropical America, 39% in tropical Africa and 37% in tropical Asia are dominated by Oxisols and Ultisols representing 1.6 billion hectares (Sanchez and Salinas, 1981) which have typically low pH and a high aluminum saturation. Aluminum comprises 7.1% by weight of the earth's crust (Lindsay, 1979). Aluminum in soil comes from minerals such as feldspars and micas which weather and release Al. These are converted to aluminosilicate minerals and they in turn result in aluminum oxides and hydroxides due to chemical weathering and breaking down. At pH 5.0 and below, soluble Al is released into the soil water. The amount of soluble Al is dependent on soil pH, amount of minerals such as feldspars and aluminosilicates in the soil, exchange equilibrium with exchange surfaces and reaction with organic constituents in forming complexes (Bell and Edwards, 1986). At soil pH 5.0 and below, soluble Al is considered to be the most important growth limiting factor (Foy, 1988).

1.2.3.2 Soil aluminum toxicity

Soil testing methods to detect the amount of aluminum toxicity are not very efficient because of both toxic and non-toxic forms being present in the soil solution. Aluminum exists in a variety of forms and various plant species differ in their toxic reactions to these species. Moreover the presence of inorganic and organic ligands, variation in soil moisture and temperature, variation in soil exchange surfaces with time and space, influence of plant roots in the rhizosphere, overlapping effects of nutritional deficiencies and other elemental toxicities all make soil testing for aluminum toxicity a difficult task. Commonly used methods include measurement of soil pH, extraction of exchangeable Al, assay of soil Al saturation, and chemical extraction of soil Al. Further research is needed to devise better

methods to determine toxic Al components in the soil (Wright, 1989). Since the ionic charge of aluminum and the crystalline radius are both high, the aluminum ion is very active in solution. The charge of the aluminum ion varies with the pH of the soil solution. When a mineral containing aluminum dissolves it releases Al^{3+} which forms coordinate bonds with six OH_2 groups. As the acidity of the soil solution decreases, the OH_2 groups dissociate step by step to give products such as $Al(OH)^{2+}$, $Al(OH)^{+}_2$, $Al(OH)_3$ and $Al(OH)^{-}_4$ respectively at increasing pH (Bell and Edwards, 1986). As the OH:Al ratio increases, polynuclear hydroxy-Al species are formed which are metastable intermediates in the precipitation of solid phase $Al(OH)_3$. Several inorganic ligands such as fluorine and sulfate radical and organic ligands form complexes with aluminum. The phytoxicity of these complexes has been studied using short-term solution culture studies (Parker et al. 1988; Parker et al. 1989; Kinraide and Parker, 1987; Alva et al. 1986; Cameron et al. 1986; Hue et al. 1986). It was found from these studies that non-toxic species were Al-SO_4 (Kinraide et al. 1987; Alva et al. 1986; and Cameron et al. 1986), Al-F (Cameron et al. 1986), and organic complexes (Hue et al. 1986) of aluminum. A polynuclear hydroxy Al species (Al_{13}) was toxic in artificial media but its role in natural soil systems has not yet been elucidated. Al^{3+} is considered to be the most phytotoxic species of mononuclear Al species (Parker et al. 1989). $Al(OH)^{+}_2$ and $Al(OH)^{2+}$ have also been claimed by some workers to be toxic (Alva et al. 1986). However in studies in wheat it was shown that the Al^{3+} was toxic and not the other two species (Kinraide and Parker, 1989). In dicotyledonous plants mononuclear Al hydroxy species were seen to be harmful rather than Al^{3+}. It might not be possible to clearly indicate separately the toxicities of mononuclear species without producing errors by expressing hydroxy Al-monomers as a function of the activities of Al^{3+} and H^{+}. Attempts to relate root growth to Al speciation in soil solution in the absence of polynuclear Al have shown that Al^{3+} and perhaps $Al(OH)^{2+}$ and $Al(OH)^{+}_2$ are involved. The toxicity of mononuclear hydroxy-Al species is generally attributed to Al^{3+} which is influenced by the solution pH (Kinraide and Parker, 1989).

1.2.3.3 Liming acid soils

Treatment of acid soils with lime ($Ca(OH)_2$) can be a solution for acid soil regions where crops such as wheat are grown. Liming has several advantages. It affects nitrogen use efficiency. An acid soil pH provides a favorable condition for mineralization reactions of organic N due to which there is an increase in mineral N. It has been reported that the correlation of mineral N with soil pH is more (r=-0.61 **) than with log_{10} exchangeable Al (r= 0.42 *) in ultisol sites (Nyborg et al. 1988). Al toxicity could be a factor in reducing

mineral N formation from organic N under conditions of low pH (Adams and Martin, 1984). Liming acid soils helps to increase soil pH. Addition of Al complexing ligands have been shown to decrease Al toxicity (Kinraide and Parker, 1987, and Parker et al. 1988). Liming is considered the most common method of overcoming soil acidity but other methods such as addition of Al complexing ligands (SO_4, F) (Sumner and Carter, 1988) and organic ligands (Ahmad and Tan, 1986) have also been suggested. Addition of calcium and other cations to nutrient solution can reduce Al toxicity by increasing the ionic strength of the solution and hence reducing the concentration of Al^{3+} ion (Kinraide and Parker, 1987). Reports have suggested that boron addition could alleviate aluminum toxicity by improving growth, nutrient uptake and by affecting uptake of B and Al in acid soils (LeNoble et al. 1991) but it was found that upon addition of boron to nutrient solutions containing aluminum that there was increased uptake and immobilization in roots without a significant influence on growth (Taylor and Macfie, 1994). Sometimes liming is neither an economic nor a completely effective method of soil correction in acid aluminum toxic soils. The reasons are the high cost of transportation of lime to field sites, and lack of efficient technology for changing the soil pH below the incorporation depth in the top soil layer. Both reasons are very valid in developing countries where transportation is often difficult and costly, and neither application technology nor lime may be readily available. Therefore, a combination of breeding and agronomic measures would jointly help to solve the problem of toxicity (Fisher and Scott, 1993).

1.2.4 Aluminum toxicity

Symptoms of aluminum toxicity are dramatic in root and shoot growth. The symptoms resemble phosphorus deficiency, late maturation of leaves which results in the formation of small green leaves, purpling of stem, leaves, leaf veins, chlorosis and necrosis in leaves (Foy, 1984; Foy and Brown, 1963; Alam and Adams, 1979; Jarvis and Hatch, 1986; Unruh and Whitney, 1986). Aluminum toxicity decreases total chlorophyll concentration in the leaf and the rate of photosynthesis in wheat, but the decrease in the transpiration is the most severe (Ohki, 1986). Roots are more affected by toxicity than shoots. There are other symptoms such as petiole collapse and mottled chlorosis, which are typical of Ca^{++} deficiency (Armiger et al. 1968), and interveinal chlorosis which resembles Fe deficiency (Taylor and Foy, 1985). The roots become shortened and thickened, stubby, brown in color, and brittle, and occasionally necrosis can be seen in the roots. Lateral root growth is less but initiation of lateral roots close to the apex of the main root axis is seen. The root system is not finely

branched and is greatly reduced in size and is coralloid in appearance (Foy, 1984; Clarkson, 1965; Fleming and Foy, 1968; Kesser et al. 1975; Kesser et al. 1977). The effect of Al stress on root development makes the plant susceptible to drought and produces secondary responses which are harmful to the overall growth of the plant. In barley it has been demonstrated that aluminum stress increased the effect of water-stress (Krizek and Foy, 1988). Absorption of relatively immobile elements such as P is reduced due to a reduction in absorption and root surface area, or a damaged plasma membrane. Deficiencies of elements such as P, Ca, Mg, Fe, and Mn could be collectively caused (Taylor and Foy, 1985). Though the extent of research directed towards understanding the physiological and biochemical effects of Al on plants has been very intense in the past few decades there is still a lack of complete understanding of the exact process by which toxicity occurs in the plant. Nevertheless, numerous mechanisms for tolerance have been proposed.

In recent reviews a consensus has arisen that Al-toxicity response occurs at the cellular level (Cumming and Taylor, 1990; Haug, 1984; Taylor, 1988, 1991). The disorganization of the plasma membrane is the first symptom of aluminum toxicity stress. Since the most easily recognizable signs occur in the root, the root cap, meristem and the elongation zone have been flagged as important primary sites for toxicity to occur (Bennet and Breen, 1991). Aluminum accumulates in greater amounts in the root apex and the surrounding mucilage, and the damage caused in this region is more than in other parts of the root containing mature tissue (Fleming and Foy, 1968; Horst et al. 1982, 1983; Bennet et al. 1985; Wagatsuma et al. 1987; Rincon and Gonzales, 1992; Taylor et al. 1996). There is an arrest in the rate of mitosis (Clarkson, 1965; Horst et al. 1982, 1983; Matsumoto and Morimura, 1980; Morimura et al. 1978) because of accumulation of Al in the apex, which is made up of dividing cells. Al has been found to bind with DNA in vitro and in vivo and this has been suggested to be the reason for a decrease in the rate of mitosis (Matsumoto et al. 1976; Morimura et al. 1978; Wallace and Anderson, 1984). Under Al stress, cells in the root cap have been observed to become vacuolated, showing disruption of Golgi body function and plastid development, with changes in the structure of the nucleus, loss of cytoplasm, and other structural disintegration. Epidermal, endodermal, and cortical cells of aluminum affected plants rapidly autolyze, and become swollen or disrupted. The meristem becomes so disorganized that the differentiation between the root cap and vascular system cannot be recognized. The response to aluminum is not the same in all species of plants. Existence of differential genetically controlled tolerance to aluminum within several species has been reviewed (Taylor, 1988).

1.2.4.1 Uptake and distribution of aluminum

Several factors such as availability of Al ions, pH of the soil solution, ionic strength of the growth medium, presence of chelating ligands and the plant genotype all influence the toxicity symptoms produced by the plant (Pavan and Bingham, 1982; Blamey et al. 1983; Kinraide et al. 1985; Wagatsuma and Ezoe, 1985; Alva et al. 1986; Alva et al. 1986 (a, b); Hue et al. 1986). The identity of the form of the toxic aluminum ions is not yet known definitely but since many trivalent metal ions are known to be toxic to plants and since aluminum toxicity is found in acid soil regions, Al^{3+} is thought to be the major phytotoxic species. Whether Al^{3+} enters through the plasma membrane is an important question. Even though polyvalent ions such as Al^{3+} are insoluble in the lipid bilayer of the plasma membrane it has been found that almost half the amount of total Al in plants entered into the symplasm (Tice et al. 1992). This could have taken place with the help of membrane bound proteins, via stress-related lesions. Aluminum tolerant genotypes accumulate less Al than sensitive genotypes. In a recent study aluminum was detected in the symplasm after 30 minutes of exposure (Lazof et al. 1994). Upon entering the symplasm aluminum can bind with important molecules involved in metabolism (Haug, 1984; Martin, 1988; Haug et al. 1994). Aluminum can enter the apoplasm. Upon entering the apoplasm aluminum could bind to pectin residues of protein molecules in the cell wall and reduce extensibility and hydraulic conductivity, remove critical ions from their sites of attachment to the cell wall, bind to the lipid bilayer or membrane-bound proteins and thus disrupt activities which are essential for metabolism. This could trigger a secondary-messenger pathway (Haug, 1984; Taylor, 1988; Bennet and Breen, 1991; Rengel, 1990: Haug et al. 1994). That the apoplasm is a site for aluminum toxicity has been shown (Ownby and Popham, 1989). The apoplasm might not be very permeable to aluminum as was seen in some experiments with maize in which the layers outside the endodermis did not always show a localization of aluminum accumulation (Rasmussen, 1968). The root cap and mucilage surrounding the roots however shows the highest concentration of accumulation, but with increasing time aluminum is seen to reach the stele (Bennet et al. 1985). It seems likely that the root apoplasm presents a barrier to the uptake of aluminum into the other parts of the plant. In soybean and maize it was demonstrated that by excising the roots there was an increase in uptake of aluminum in the leaves and there were other symptoms such as decrease in water uptake, and aluminum toxicity symptoms which are not normally noticed (Wagatsuma, 1984). Generally the leaves do not show any response in terms of concentration of accumulated aluminum due to toxicity

(Mugwira et al. 1976) until the binding sites in the root are saturated (Wagatsuma, 1984). The ability to exclude aluminum from shoots and roots appears to be a tolerance mechanism (Foy and Peterson, 1994; Taylor et al. 1996).

1.2.4.2 Physiology of aluminum toxicity

Reduction in growth in wheat is an effect of aluminum toxicity. The primary site of damage is the root which becomes stunted, swollen, discolored and lacks fine branching and root hair formation. Several reviews have been written about the various types of injury caused by aluminum (Foy et al. 1978; Bergmann, 1992). It has been observed that the inhibition of wheat root growth by aluminum occurs after a minimum lag period of growth which could be as short as two hours (Bennet and Breen, 1991; Ownby and Popham, 1989; Ryan et al. 1993). Parker (1995) observed that aluminum toxicity effects occur at two levels. The immediate reaction to toxic aluminum is an 'acute' reaction and can be observed during short term experiments. The initial 'acute' inhibition of growth is later followed by a 'chronic' inhibition. This was observed in experiments (Parker, 1995) in which 'Scout 66' (a sensitive cultivar) became acclimated to low levels of aluminum, and resumed growth after the initial period of inhibition of growth, similar to the tolerant cultivar 'Atlas 66'. No correlation was found however between acclimation and tolerance. 'Acute' inhibition might be seen in physiological experiments which are for a short time period and the 'chronic' toxicity could be observed in long-term field tests. Therefore, it was suggested that measures of 'acute' toxicity tolerance using methods such as root growth (Kerridge et al. 1971) and hematoxylin staining (Polle et al. 1978) might not be very accurate for selecting the best varieties for the field (Carver and Ownby, 1995). Nevertheless root growth assays and hematoxylin staining still continue to be popular among breeders because they are quick and easy methods, readily carried out under conditions of aluminum stress at low pH in hydroponics.

The root accumulates more aluminum than other mature tissues and shows greater visible damage. It has been shown that only 2 to 3 mm of the root apices have to be exposed to aluminum in order to cause a reduction in growth (Ryan et al. 1993). When the entire elongation zone except the root apex is exposed to aluminum it has been seen that there is no inhibition of growth (Ryan et al. 1993). Thus the root apex was thought to be involved in the inhibition of root growth. Experiments later demonstrated that the onset and extent of inhibition were the same in capped and decapped roots of maize (Ryan et al. 1993). This result then questions the hypothesis that the root cap itself is directly involved in inhibition of growth by aluminum. It is clear that the root cap has an important role to play in growth

inhibition even though it might not be directly involved in the toxicity mechanism. Within the root meristem and root cap cells there is an increase in vacuolation and production of starch grains (de Lima and Copeland, 1994) and disruption in dictyosomes and their secretion (Bennet et al. 1985; Puthota et al. 1991).

The kinetics of aluminum uptake in wheat roots was studied by Zhang and Taylor (1989) who found that there was a minimum binding period of 30 minutes after which there was a linear period of uptake. The magnitude of the uptake during the linear period was the same for both sensitive and tolerant cultivars. Later they found that during the linear period of uptake two processes occurred: fixation of aluminum in the apoplast and the entry through the plasma membrane (Zhang and Taylor, 1990). In several studies it has been seen that tolerant plants accumulate less aluminum than do sensitive cultivars and this has been seen in roots, especially in the zone of elongation and cell division (Rincon and Gonzales, 1992; Delhaize et al. 1993). Detection studies of aluminum deposition in various parts of the root using X-ray microanalysis have not produced consistent results. Naidoo et al. (1978) showed that aluminum was found in the nucleus. Using better techniques aluminum was only detected in the cell wall. However, using X-ray microanalysis methods on samples treated for 24-hours with aluminum, researchers could not find any detectable levels of aluminum (Ownby, 1993; Marienfeld and Stelzer, 1993). Other methods such as hematoxylin (Rincon and Gonzales, 1992) and fluorochrome morin staining (Tice et al. 1992) could detect aluminum in the nucleus of wheat root cells. Tice et al. (1992) also concluded that about 55 to 70% of the total aluminum accumulates in the symplast after a period of 48 hours of exposure.

Aluminum toxicity is thought to produce nutrient deficiencies of certain essential elements such as phosphorus and calcium and to a lesser extent magnesium and nitrogen. Whether this secondary effect forms any of the basis of toxicity is unclear because these deficiencies express much later than other symptoms such as root growth inhibition. Moreover, the sites of these deficiencies are far removed from the initial sites of toxicity. The appearance of toxic symptoms is thought to be a secondary reaction or effect of toxicity. Andrew et al. (1973) found that Al-tolerant legume species can better manage nutrient deficiencies under aluminum stress than sensitive cultivars. It could be that since aluminum disrupts membrane function it reduces the uptake and accumulation of minerals. ATPase activity was inhibited by aluminum in root extracts of *Hordeum distichon* both *in vitro* and *in vivo* (Veltrup, 1983). Aluminum toxicity effects are seen on phosphate metabolism (Hanson

and Kamprath, 1979; Klimashevskii and Bernatskaya, 1973; Klimashevskii et al 1970; Clarkson, 1965; Clarkson, 1969; Pfeffer et al. 1986). In barley roots treated with Al, the phosphorylation of sugar was inhibited by Al. It was observed that there was an increase in the amount of ATP and other nucleotide phosphates and this suggests that rate of ATP synthesis is not affected by toxicity (Clarkson, 1965; Clarkson, 1969). Aluminum can bind with ATP forming stable complexes (Neet et al. 1982) and this inhibits the activity of yeast hexokinase. This inhibition is reversed by the addition of phosphate or chelating ligands. It could explain why aluminum inhibits the ATPase activity in pea roots (Klimashevskii et al. 1970). Toxic effects of aluminum also occur in the shoot, affecting various metabolic functions. In wheat, reduction in growth, chlorophyll content, and transpiration were each associated with increasing aluminum toxicity (Ohki, 1986).

1.2.5 Aluminum toxicity tolerance

1.2.5.1 Physiology of tolerance

Tolerance to aluminum has been reviewed in several species (Taylor, 1988) and there exists a differential tolerance to toxic levels of aluminum in various species. The variability in tolerance to aluminum found between and within species forms the basis of plant breeding efforts to develop varieties suitable for acid soils. Tolerance to aluminum can be grouped into exclusion mechanisms and internal tolerance mechanisms. This grouping is based on the site of metal detoxification or immobilization, or the site where there is adaptation to aluminum stress. Exclusion mechanisms take place in the apoplasm and internal tolerance mechanisms are found in the symplasm.

In an external exclusion mechanism aluminum is secluded in the apoplasm, thus preventing it from entering the symplasm. The extent to which aluminum is stopped from entering the symplasm is not known. The cell wall seems to play a large role in the external tolerance mechanism. The cell wall could be a sink for the aluminum that enters into the root. Al-tolerant cultivars take up less aluminum than Al-sensitive cultivars (Delhaize et al. 1993; Rincon and Gonzales, 1992; Tice et al. 1992). However it is not known whether the tolerant plants exclude more aluminum than sensitive ones. Aluminum uptake into the apoplasm seems to be related to root cation exchange capacity, CEC (Wagatsuma, 1983, 1984). In wheat, aluminum uptake by aluminum-tolerant plant roots (low CEC) was less than the uptake by aluminum-sensitive plants (high CEC). CEC of excised roots of plants of other species did not however show such a clear cut difference (Huett and Menary, 1979). The plasma membrane is selectively permeable and this could form a barrier against the entry of

aluminum into the symplasm. The plasma membrane has been shown to act as a barrier against the movement of aluminum into the cytosol (Wagatsuma, 1983) but the effectiveness of the tolerance mechanism is reduced when metabolic activity rates are low. Sufficient data do not exist to suggest that this could be the only barrier but since the plasma membrane differentiates the symplasm from the apoplasm its selectivity could be a mechanism for tolerance to aluminum.

Exudation of chelating ligands in the root zone which results in formation of stable complexes between aluminum and the chelating molecules could act as an exclusion mechanism taking place in the rhizosphere or it could also take place internally. Adding citric and malic acid to cell cultures of sensitive cells reduces the toxicity effect (Ojima and Ohira, 1982). Secretion of malic acid in response to aluminum in the culture medium has been postulated to be a mechanism for chelating aluminum in solution (Delhaize et al. 1993b; Ryan et al. 1995) and this showed strong correlation with solution monomeric aluminum concentration. Differential secretion of malate in wheat genotypes was reported by Basu et al. (1994b) and Ryan et al. (1995). Even though there is a high correlation between aluminum tolerance and malate exudation there is no evidence to show whether the malate produced is sufficient to detoxify the aluminum in the root tip by reducing the aluminum concentration at least adjacent to the cell membrane. It has been seen that aluminum-tolerant tissue produces more citrate molecules than the sensitive plant tissue (Miyasaka et al. 1991; Ojima et al. 1984; Pellet et al. 1995). Citrate has been seen to form a strong complex with Al^{3+} and is more effective in correcting toxicity than either succinate or malate (Ownby and Popham, 1989). The exudation of organic acids is one among the many mechanisms of tolerance which have been extensively reviewed (Taylor, 1991).

Several genes in wheat have been sequenced and their protein products studied to understand their role in the tolerance to aluminum (Snowden and Gardner, 1993; Richards et al. 1994). The proteins encoded by the cDNAs show structural similarity to metallothionein-like proteins (wali 1), but their role in the tolerance mechanism could not be clearly defined because they are induced much later than when toxicity first affects the plant. They could however be part of the tolerance mechanism to several metal stresses (Basu et al. 1994a).

In certain wheat cultivars the external tolerance mechanism seems to be working whereas in plants such as tea, where leaves accumulate high levels of aluminum without showing toxicity symptoms, there could be an internal tolerance mechanism operating. In comparison to the amount of research activity devoted towards the study of exclusion

mechanisms there has been relatively less study of internal tolerance mechanisms which could take place in the cytosol. The higher amounts of organic acids in aluminum tolerant cultivars than in aluminum sensitive ones could be a mechanism for tolerance, but whether this is a primary tolerance mechanism is still not clear (Taylor, 1991). Another internal tolerance mechanism could be the sequestration of aluminum in relatively less-sensitive sites in the cytosol, such as the vacuole (Taylor, 1988). There is also an increasing number of reports on metal-binding proteins, the phytochelatins, which are induced by various metal toxicities. It has been suggested that they might have a role in metal ion-homeostasis or metal tolerance (Reddy and Prasad, 1990; Tomset and Thurman, 1988). Induction of aluminum tolerant enzymes serving in competitive inhibition during the uptake of ions such as Mg^{2+} has been suggested as a tolerance mechanism (Rengel, 1990) but this hypothesis requires more evidence to be considered as a possible mechanism for tolerance. The increase in enzymatic activity such as that of NAD kinase (Slaski, 1990) could play a role in aluminum tolerance in addition to aluminum tolerant enzymes.

The understanding of the physiology of stress tolerance in plants could be further advanced by the use of stress tolerant and stress sensitive plants preferably of close genetic makeup (such as near-isogenic lines) of the same species (Foy, 1983). The near-isogenic pair could be studied to indicate various differences which lead to better understanding of perfomance of aluminum tolerance genes in soils of varying pH and aluminum toxicity level.

1.2.5.2 Genetics of tolerance

Despite the complexity in the physiology of aluminum toxicity and tolerance mechanisms the genetic control of tolerance appears to be relatively simple. In Brazil where most of the cultivated area is under acid soil there is a wide range of tolerance to aluminum in Brazilian wheat varieties (Foy et al. 1965). These cultivars still remain the standard of performance under aluminum toxicity, probably because of the selection pressure they were subjected to during their development. It was also reported that many varieties developed in the acid soil regions of the U. S. A. possess a favorable tolerance reaction to aluminum, unlike varieties which were developed in non-acidic soils. Variety classification for aluminum tolerance has been carried out in wheat (Briggs and Nyachiro, 1988; Foy et al. 1965; Foy and daSilva 1991; Mesdag and Slootmaker, 1969; Zale and Briggs, 1988) and it appears that the majority of the high-yielding cultivars which have their origin or part of their parentage from rye or wheat varieties from Brazil, Mexico, or Kenya are tolerant to aluminum toxicity. The standard of Al tolerance among winter wheats is Atlas 66 but some

spring wheat cultivars may have even surpassed Atlas 66 in their tolerance to aluminum (Briggs et al. 1989). However, most economically important Canadian Hard Red Spring Wheat cultivars were not tolerant to aluminum (Carver et al. 1988; Zale and Briggs, 1988). A recent study with near-isolines of Chisholm (Al-tolerant) and Century (Al-susceptible) indicated that there was one gene for aluminum tolerance transfered from Atlas 66 but the expression of tolerance was not as high as that found in Atlas 66 (Johnson et al. 1997). In barley, the tolerance to aluminum in some populations seems to be controlled by a single major dominant gene without maternal inheritance (Minella and Sorrels, 1992; Reid, 1971). In wheat too some reports show a single gene controlling tolerance (Kerridge and Kronstad, 1968). Other studies have indicated there might be one or three major genes, with some modifier genes (Berzonsky, 1992; Camargo, 1981, 1984; Campbell and LaFever, 1981). In the highly aluminum tolerant Brazilian cultivar BH 1146, the tolerance gene is located on chromosome 4 of genome D (Lagos et al. 1991). Studies on Redcoat and Arthur (sensitive) and Seneca and Thorne (tolerant) show that sensitivity is controlled by a single recessive gene while tolerance is a polygenic character (Campbell and LaFever, 1981). Fisher and Scott (1983) have shown that tolerance is due to a major dominant gene. Aniol and Gustafson (1984) have determined the chromosomal location of tolerance genes in wheat using nullisomic-tetrasomic and ditelosomic lines of the cultivar Chinese Spring. Tolerance genes were found on chromosome arms 6AS, 7AS, 2DL, 4DL, 7DL in wheat and 6RS, 3R and 4R in rye. Another tolerance gene on chromosome 5D has been found by Elliot (1986). Segregation studies on protein expression using near-isogenic lines for aluminum tolerance (Katepwa/Alikat) indicate the presence of a single gene (Somers et al. 1996; Somers and Gustafson, 1995).

1.2.6. Genetic improvement

1.2.6.1 Breeding for tolerance

Breeding for aluminum tolerance in Brazil probably started in the 1920s when scientists noticed the phenomenon called 'crestamento' which in Portuguese means 'burning' or 'toasting'. By 1942, the problem was explained as occurring due to soil acidity and the toxic effect was later found to be due to excess aluminum (Araujo, 1948, 1949). The cultivars Predulio and Carazinho were released in 1956 and 1957 respectively. By the late 1960s collaborative research and an active breeding program was started with CIMMYT to combine the aluminum tolerance trait with the high-yielding capacities of Mexican cultivars. Later on the shuttle program for the exchange of germplasm enabled testing and screening

for aluminum tolerance in the acid soils of Brazil and the varietal evaluation of other traits under non-acid Mexican soil (Rajaram et al. 1991). The major gains of the CIMMYT breeding program have been development of aluminum tolerant germplasm, increased phosphorus uptake efficiency, resistance to leaf spot diseases such as *Septoria, Helminthosporium*, and *Fusarium* spp. and the stay-green effect (Rajaram et al. 1991). In alfalfa, (*Medicago sativa* L.) it has been reported that *in vitro* culture and selection using a modified Schenck and Hildebrandt (SH) (1972) medium instead of hydroponic culture was successful both for embryo regeneration from callus tissue and in selecting for tolerance (Kamp-Glass et al. 1993).

1.2.6.2 Methods for screening genotypes

Selection methods for aluminum tolerance are many (Little, 1988). Use of hematoxylin dye for visual assessment of toxicity (Polle et al. 1978) is an inexpensive, simple method which could be used to screen large populations and to select a wide range of tolerance groups. Since this is not a direct measure compared to a trait such as root growth, it is not very reliable at times. Shoot growth is not always correlated to root growth (Zale, 1987). Root growth in solution culture is a better method in this respect. However this is not suitable for a very large number of genotypes, for simulation of real rhizosphere conditions. This problem arises out of lack of proper knowledge of the phytotoxic species of aluminum, complexity of ionic forms of aluminum, and the difficulty in measuring soil aluminum. The cause of the stress should be clearly defined so that a good control is obtained, otherwise selection may be found to be useless at a later stage. A recently developed laboratory screening method involves the production of callose in aluminum tolerant and sensitive seedlings (Schreiner et al. 1994; Zhang et al. 1994). The callose deposition occurs due to membrane injury (Kauss, 1989) which is caused by aluminum (Wagatsuma et al. 1987). The callose deposition in the root tip takes place within minutes after exposure to aluminum (Zhang et al. 1994). The aluminum sensitive plant produces greater amounts of callose than the aluminum tolerant ones but the regression between callose formation and root growth inhibition indicated that the same amount of callose is produced in both tolerant and sensitive plants when the root inhibition is the same. This probably means that callose deposition is an indicator of the degree of sensitivity to toxic aluminum (Zhang et al. 1994). The application of biotechnological methods has also been applied to aluminum tolerance screening. Genetic markers have been used to identify polypeptides which are produced due to stress (Somers et al. 1996). Selection for tolerance in acid soil *in situ* or in greenhouse or growth chamber

experiments could be another option. The majority of the acid soil tolerant varieties are bred in regions where the soil is acid. This factor consciously (Little, 1988) or unconsciously (Mugwira et al. 1981) presents a selection pressure on the population. This would be the most effective method for screening, however cost and site accessibility constraints limit the use of such a method. Moreover, the identification of the specific stress factor in the soil of the field site is necessary (Briggs et al. 1991). Other problems such as soil variability, flooding, drought, lodging, pests of crops such as pathogens, and animals pose difficulties.

These factors reduce the control that can be achieved compared to a solution culture test. The use of acid soil medium in greenhouse pot experiments proved to be effective with wheat (Briggs and Taylor, 1994). The choice of a particular screening method depends upon the stage of selection. During the early stages of the breeding program, a quick selection method such as hematoxylin staining (Polle et al. 1978) and seedling germination tests could be performed which could present a high selection pressure on a large plant population (Duncan, 1988). Since every method has its inadequacies and disadvantages a second method such as a field trial should be used to verify the initial screening results (Duncan, 1988). Besides initial screening, evaluation of performance of a genotype for a particular environment is more efficient if field trials are conducted under the specific stress conditions. In short, the choice of a screening method depends upon the material to be screened, i.e., the germplasm for identifying suitable parents, the size of the segregating population, or the stage of selection (Carver and Ownby, 1995). When different sources of Al tolerances are to be used in breeding programs, prior characterization of their performance in soil systems, over a range of soil pH levels and Al stress levels, is desirable.

1.2.6.3 Responses to toxic aluminum in soil and non-soil media

Responses of several species of plants to aluminum have been tested using soil media and non-soil (i.e. nutrient solution) media (Polle et al. 1978b, c; Wright, 1976). In both types of media knowledge about the precise nature and cause of stress is necessary. For example, a protocol was developed by Furlani and Clark (1981) to screen sorghum (Sorghum bicolor (L.) Moench) genotypes in which certain levels of minerals such as Al, P, Ca, Mg, K in the nutrient solution culture and certain pH levels and temperatures gave a better differential response than other combinations. In soil systems it has been observed universally that root growth is inhibited to a greater extent than leaf growth (Foy et al. 1967; Briggs et al. 1989). Shoot responses are not well correlated with root growth responses to Al stress (Zale, 1987). In non-soil media, differential production of malate, citrate, succinate, callose, metal-binding

proteins, and (speculatively) aluminum tolerance enzymes such as acid phosphatases, (Noat et al. 1980) in response to aluminum stress have been demonstrated (Section 1.2.5.1). The changes observed in non-soil media took place within minutes of exposure (Zhang et al. 1994). The minimum time to onset of plant injury in soil media has not been studied. The time and duration of exposure to aluminum are also important factors to consider. Response to exposure to aluminum is a function of plant age (Wheeler, 1994), duration of exposure (Foy et al. 1965), and concentration of aluminum in culture solution (Briggs and Taylor, 1994). Changes in protein due to aluminum stress have been expressed in the near-isogenic lines Alikat (tolerant) and Katepwa and the root growth component of this is due to a single gene (Somers et al. 1996; Somers and Gustafson, 1995). Stress response due to toxic levels of aluminum in plants could be better understood by the use of stress tolerant and stress sensitive plants, preferably of close genetic makeup (such as near-isogenic lines) of the same species (Foy, 1983). The idealized response to levels of aluminum and soil liming of tolerant and susceptible lines to aluminum toxicity has been compared to the actual response in above ground dry weight, relative root weight and seed yield (Briggs and Taylor, 1994; Briggs et al. 1991; Fisher and Scott, 1993; Foy et al. 1965; Taylor and Foy, 1985). Response of crop plants in soil to aluminum has been said to be related to exposure to additional stress factors i.e. drought stress (Goldman et al. 1989) which lead to changes in leaf area in tolerant and susceptible plants, thus producing differential effects on yield (Gan et al. 1992; Levitt, 1972). A near-isogenic pair of aluminum tolerant and susceptible genotypes is ideal genetic material to study the plant developmental responses that may lead to better performance in soils of varying soil pH and aluminum stress.

1.2.7. Alikat: background information

Soil acidity studies in Alberta were started after 1965, with the reports published in McKenzie's PhD. thesis at the University of Alberta (McKenzie, 1973; Penney, 1973) raising the general awareness of the problem. Following the assessment of all released Canadian wheat cultivars (Zale and Briggs, 1988) it was found that there was a need to develop a breeding program for acid soil tolerance in wheat. Several parental genotypes were selected for the initial breeding program and these included Maringa, PF7748, Kenya Kongoni, and Romany. The idea was to develop isogenic lines which would be aluminum tolerant. Several isolines were tested both in the laboratory and in the field. The most promising line was isoline 199 (pedigree Katepwa *3/ Maringa) which was a third generation backcross between Maringa (Al-tolerant) and Katepwa (Al-sensitive) (Briggs and Taylor, 1994). This line

possessed the tolerance of Maringa to toxic aluminum and agronomic similarity with the recurrent parent Katepwa. The study of aluminum toxicity tolerance using the near-isogenic pair of Alikat and Katepwa is advantageous over studies involving tolerant and sensitive varieties with greater genotypic difference than these two, where polymorphism for many genes unrelated to Al tolerance are expected. Several studies have subsequently shown that aluminum stress induces polypeptides in this genetic system which might have a role in differential tolerance (Basu et al. 1994a,b). The aluminum tolerance gene from Maringa (tolerant) produced in Alikat (tolerant) a polypeptide profile which was quite different from that of Katepwa (sensitive) (Somers and Gustafson, 1995). Alikat, and Maringa also accumulated more protein in their root tips than the sensitive genotype Katepwa (Somers et al. 1996). These responsive polypeptides might be usable as molecular markers for screening tolerance in the future. A root exudate protein (23kD) was found to be induced by Al^{3+} toxicity (Basu et al. in preparation) and this could be an aluminum-binding protein. Efforts are currently underway to clone the Alikat derived gene (Basu et al. in preparation). This well-characterized near-isogenic pair of Alikat and Katpewa is therefore excellent material to use to study phenotypic development patterns associated with the aluminum tolerance trait, under soil conditions with a wide range of pH and aluminum stress levels.

1.3 Questions addressed by the thesis

The literature review shows that aluminum toxicity studies have been conducted on several crops and that many suitable screening methods have been devised. Experiments using hydroponics have demonstrated differential response curves of aluminum tolerant and susceptible plants to varying aluminum levels. The near-isogenic pair Alikat (Al-tolerant) and Katepwa (Al-susceptible) have shown differences in callose production, protein production in the root tip, root growth and hematoxylin staining under Al stress in hydroponic media. There has not yet been a study on this pair of genotypes on root and shoot growth in aluminum toxic soil at a wide soil pH range which includes pH where aluminum toxicity is high (pH 4.4) up to pH 7.0 where aluminum is not toxic. Such a study would demonstrate and/or confirm the pH range under which the Al tolerance gene is effective in soil. Moreover, most studies have been done with short-term experiments and long-term toxicity effects to maturity have not been studied. Studies on plant vegetative growth and development (eg. using the Haun developmental scale) and leaf area development in the near-isogenic pair of Alikat and Katepwa were also lacking, which could be useful to

demonstrate the impact of tolerance on early plant development in soil possessing toxic Al properties.

The objectives of the thesis were:

(i) To study the root and shoot growth response curve of the Alikat/Katepwa near-isogenic pair containing a gene for aluminum tolerance grown under variable soil pH range (4.4 to 7.0) in an acid, highly eluviated, gleysol obtained from the Silver Valley region in NW Alberta, Canada.

(ii) To study the effects of long-term exposure to aluminum toxicity on seed and biomass yield in greenhouse acid soil experiments using the near-isogenic pair for aluminum tolerance, under a wide range of pH and Al toxicity levels.

(iii) To study Haun scale stages and leaf area development in Alikat and Katepwa plants as a response to soil aluminum toxicity based over time, to determine differential effects of the Al tolerance gene on early plant development under different Al stress levels.

This characterization of the growth response due to an Al tolerance gene in Alikat is useful for predicting the performance of this tolerance source when transferred to plants growing in soils of varying pH and aluminum toxicity levels, as found in the soils of Northern Alberta.

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Chapter II

Role of an aluminum tolerance gene in root and shoot growth response to varying levels of aluminum toxicity, in aluminum tolerant/intolerant near-isogenic lines.

2.1 Introduction

Aluminum is a major factor of toxicity and a major growth limiting factor in acid soils (Foy, 1988). One of the primary symptoms of aluminum toxicity in plants is a change in the physiology of the root (Taylor, 1988). It was first observed that in roots of *Allium cepa* there was a reduction in root growth after 6 to 8 hours of exposure (Clarkson, 1965). In wheat, it was observed that after 2 to 3 hours a similar inhibition of root growth occurred (Ownby and Popham, 1989; Wallace and Anderson, 1984). The variability in responses to aluminum toxicity in wheat cultivars has been reported by several workers (Beckman 1976; Briggs et al. 1989; Campbell and Lafever, 1976; da Silva, 1976; Mugwira et al. 1981; Nyachiro and Briggs, 1994; Taylor and Foy, 1985a,b). The most important toxic species of aluminum is the free trivalent ion which is available at a pH of 3.5 to 5.0 (Macdonald and Martin, 1988). The tolerance level of a cultivar can be assessed by growing the plants under varying levels of aluminum toxicity (Kerridge et al., 1971; Lafever et al. 1977; Taylor and Foy, 1985a,b). Seedling root response is a good method of screening plants for aluminum tolerance (Fleming and Foy, 1968).

Aluminum stress response experiments in nutrient culture offer advantages but these studies use high levels of aluminum, often several times higher than that found in the soil solution of acid soils and therefore are not necessarily representative of real field conditions. Pot experiments using acid soil and limed treatments have been carried out on several genotypes to devise a screening tool for the greenhouse (Foy and daSilva, 1991). Methods using both soil and nutrient culture have been developed for screening crop species for aluminum tolerance (Polle et al. 1978; Wright, 1976). Nutrient culture is often preferred because soil may contain other limiting factors which might interfere with the response. However, the use of soil in a greenhouse experiment is useful because it produces a simulation of actual field conditions which can be quite different from nutrient solution culture.

There is growing evidence which supports the idea that tolerance to aluminum toxicity in wheat occurs due to the presence of a single dominant gene. Traits which are controlled by a small number of genes are studied most conveniently by comparison of near-isogenic lines produced by backcrossing. The difference in phenotype produced by the presence of a single

gene for aluminum tolerance in the ideal situation would help further the understanding of the function of the gene and its potential value. In a study involving Canadian spring wheat cultivars (Zale and Briggs, 1988) it was seen that Katepwa is Al-sensitive. In contrast, Maringa is a Brazilian cultivar which is an Al-tolerant spring wheat standard. Efforts to produce isogenic lines which would be Al-tolerant have been ongoing in the University of Alberta wheat breeding program using Maringa and other tolerance sources (Briggs and Taylor, 1994). For the present study Alikat (Al-tolerant = Katepwa*3/Maringa) which is agronomically similar and isophenotypic to its recurring parent Katepwa (Al-susceptible) but differing in the aluminum tolerance gene were chosen. Plants were grown under greenhouse conditions in an acidic humic eluviated gleysol (pH 4.4) from Silver Valley in NW Alberta under varying but controlled levels of aluminum toxicity.

The purpose of these experiments was to investigate the pH range within which the aluminum tolerance gene would affect the growth of the root and shoot. A response curve of the two near-isogenic lines across a range of variable aluminum toxicity was sought. The response in root and shoot weight to variable aluminum toxicity levels in a soil system conditioned by pH was also studied. Since Alikat (tolerant) and Katepwa (sensitive) are nearly identical to each other in all other characteristics except the aluminum tolerance trait, they were expected to show differences in root and shoot growth only in response to variable levels of aluminum toxicity.

2.2 Materials and methods

Plant material

Two near-isogenic lines for aluminum tolerance, Katepwa (sensitive) and Alikat (tolerant) were used in this study. Seeds were obtained from the University of Alberta, Experimental Research Station. Alikat was produced by crossing Maringa (Al-tolerant Brazilian cultivar) with Katepwa (a locally adapted Al-sensitive cultivar, recurrent parent) and backcrossed for three generations. This backcross product named Isoline-199 was as tolerant as Maringa to aluminum in nutrient solution up to 600 µM (Briggs and Taylor, 1994) and was isophenotypic with Katepwa in its agronomic traits. However, field trials in Edmonton in the absence of aluminum stress have shown that this near-isogenic Alikat (Isoline-199) differs from its recurrent parent Katepwa in days to heading (Briggs and Taylor, 1994). In all other agronomic traits Alikat has been seen to perform similarly to Katepwa (Briggs, 1997, unpublished data.). During experiment 1, the plants were grown in acid soil experiments conducted in the greenhouse for a period of three months until the plants reached the milk stage. In experiment

2, the plants were grown in acid soil for a month up to the time they reached the late flowering stage.

Growth medium

The soil, an acid humic eluviated Gleysol of the Josephine series used in the two experiments (1 and 2) was obtained from the Silver Valley region in NW Alberta (location 58^0 10' N, 118^0 50' W), (characterized in McKenzie, 1973), Canada. The initial soil pH was measured (1:1 ratio of soil and water w/w) according to the method described by McKeague, 1978 and was found to be 4.4 which was considered highly acidic for growing wheat. A soil pH response curve (Fig. 2.1) was prepared using $Ca(OH)_2$ as a soil amendment. Exchangeable aluminum in the soil solution was measured using an atomic absorption spectrophotometer (Perkin-Elmer model 3030) using acetylene-nitrous oxide flame (McKeague, 1978). The Al^{3+} (mg kg^{-1}) availability curve was also prepared (Fig. 2.2) which showed the response in available aluminum to pH change. The aluminum ion (Al^{3+}) was measured because at the soil pH range of 4.4 to 5.5 this ion is considered most toxic to plants (Marion et al. 1976). Total soil nitrogen measured was 770 µg g^{-1}, and total phosphorus measured was 307.3 µg g^{-1} while available phosphorus was 3.5 µg g^{-1}. A recommended supplemental dose of 20-20-20 all-purpose fertilizer (Plant Prod) at 3g l^{-1} was applied fortnightly. The fertilizer source was composed as follows: Total Nitrogen (N) 20%; Available phosphoric acid (P_2O_5) 20%; Soluble potash (K_2O) 20%; Boron (B) (actual) 0.02%; Chelated copper (Cu) (actual) 0.05%; Chelated iron (Fe) (actual) 0.10%; Chelated manganese (Mn) (actual) 0.05%; Molybdenum (Mo) (actual) 0.0005%; Chelated zinc (Zn) (actual) 0.05%; EDTA (ethylene diamine tetraacetate) (chelating agent) 1.0%. Ten treatments were made by adding varying levels of $Ca(OH)_2$ to the soil. The soil treatments were mixed before they were poured into plant tubes. After mixing the lime in the soil it was left to incubate for one day before the seeds were sown in the soil. The soil pH was measured after the addition of slaked lime by using 1:1 w/w soil to water ratio.

Plant culture

Cylindrical root growth tubes (52 cm long, and 10 cm in diameter, cut in half) were filled with equal amounts of soil (1000g) and placed vertically in trays which were watered twice a week. The tubes had been cut in halves along the length and a rectangular plexiglass cover was attached on the flat side of the cut cylinder. This would ensure that during the time of harvest there would be no loss in the sample collection and in separating the plant material from the soil. The plexiglass cover was opaque to ensure that the root growth would not be affected by sunlight. The tubes were filled completely to the brim with soil so that there would not be any

shading effect on the growing plants which could result in experimental error. The tubes were sealed with tape to prevent any loss of soil during watering or the growth of roots outside the soil column. The trays had holes at the bottom through which excess water could be drained out at regular intervals. Seeds of similar size were chosen and were directly sown in the soil 2 cm below the surface. The experimental unit was one plant per tube filled with soil. Watering was done after seed sowing to promote the germination process and moisture status was maintained at field capacity through regular watering. The total photoperiod hours were 16. Plants were supplemented with H. I. D. (High Intensity Discharge) lamps H. P. S. (High Pressure Sodium) 400W Sylvania lamps at a range of approximately 450 $\mu Em^{-2}s^{-1}$. Light fell vertically from the light source 1 meter above plant height. Temperature in the greenhouse was maintained at 21^0C by an emergency vent set at 23^0C.

Bioassay

The experimental design was a randomized block design (2 x 10 factorial) with ten soil treatments, two cultivars, and four blocks as replications. In experiments 1 and 2 day length periods were 16 hours and temperature of the greenhouse chamber was maintained at 69 ^0F. After the completion of the experiments 1 and 2 (90 and 30 days after planting, respectively) soil columns were opened and the soil was washed away carefully, the plants were harvested and relevant measurements made. Plant shoots and roots were collected separately and oven dried at 60^0C for 12 hours. Dry weights of shoots and roots were measured separately.

Data analysis

Data were analyzed by the Analysis of Variance Procedure of the Statistical Analysis System (SAS Institute). Curve fitting analysis of data across several pH levels was done using the Repeated Measures Analysis of Variance (Petersen, 1985). The separation of means was done using Fisher's least significant difference criterion based on Student's t distribution.

2.3 Results and discussion

The results obtained in experiment 1 (Fig. 2.3) were considered as not optimal because it was learned during the process that the degree of control was not very stringent. During the end of the three month period (ninety days after sowing (DAS)) the plant roots grew longer than expected (probably due to hydrotropism) and eventually were seen to be growing out of the bottom of the root tubes into the water bath. Thus it was not very clear whether the aluminum toxicity factor was present in the root tip regions which were emerging into the water bath in which the tubes had been placed vertically. The water bath contained the fortnightly dose of nutrients and no aluminum. Therefore the lower portion of the roots grew

rather luxuriantly instead of retaining any residual effect of aluminum toxicity which was present in the soil media within the column. The experiment was repeated but it was conducted for a shorter period of thirty days after sowing (DAS) only.

The data presented here is from experiment 2 which was conducted under the same conditions as experiment 1 except for the difference in the duration of the experiment which was conducted until only thirty days after sowing (DAS). The mean values of root, shoot and total biomass dry weight of the aluminum tolerant Alikat and the sensitive variety Katepwa in pots containing Silver Valley soil which had been pH-controlled to change the level of aluminum toxicity by the addition of slaked lime (Fig. 2.4). The vertical bars indicate standard error of mean for each treatment. It can be seen that standard errors of shoot and total biomass dry weights are larger in comparison to those for root dry weight. Moreover, the standard errors for root, shoot and total biomass dry weights at soil pH values higher than 6.0 are much higher than for those at pH values below 5.5. This could be important for understanding the action of the particular aluminum tolerance gene in question. The protocol used in this experiment was conducted using the identical system as in a prior study done by Briggs et al. (unpublished data) in which the current problems did not occur. The problem could not be detected until root emergence from the root tube occurred, at which time it was too late. Due to this effect smaller differences would be expected between the near-isogenic lines.

The analyses of variance for root, shoot and total dry weights of plants at variable aluminum toxicity levels were done for all three parameters and it was found that the pH treatment and variety effects were significant. The pH treatment x variety interaction however was significant only for total dry weight data. The fraction (R-square) of total variation in the data explained by the experimental model was the highest for total biomass ($R^2 = 0.56$) but for the root weight data it was almost near that value ($R^2 = 0.52$) whereas for shoot weight data it was lower ($R^2 = 0.49$). This could be stated as similar for all three measurements.

For the next part of the data analysis the root weight data at ten levels of aluminum stress denoted by ten soil pH levels, were analyzed using repeated measures analysis of variance (Table 2.3) to test the initial objective of the experiment. Another repeated measures analysis of variance (Table 2.4) was done by partitioning the data between the pH range of 4.4 to 5.5 to observe whether the differences were magnified when one used the data from pH range where Al tolerance is expected to be effective. The purpose was to see whether the aluminum tolerance gene produced a differential effect on root growth at pH above 5.5 at which there was theoretically no aluminum toxicity. The repeated measures analysis of variance (Table2.4)

indicated that the best-fit curves for the varieties were not significantly different. This was probably because low number of data points reduced the sensitivity of the curve-fit process.

Table 2.1. Anova of root, shoot, and total plant dry weight showing mean squares (MS) of Alikat and Katepwa growing in variable acid gleysol from Silver Valley for thirty days (Experiment 2) shown in Figure 2.4.

Effect	df	Total weight (MS)	Root weight (MS)	Shoot weight (MS)
Block	3	0.021	0.002	0.024
pH	9	0.214**	0.011**	0.108**
Variety	1	0.437**	0.022**	0.209**
pH*Variety	9	0.056*	0.002	0.040
Error	57	0.040	0.002	0.029

** indicates significant difference at 1% level; * represents significant difference at 5% level.

Table 2.2. Repeated measures analysis of variance showing mean squares (MS) for root weights of Alikat (tolerant) and Katepwa (sensitive) growing under ten levels (pH range of 4.4 to 7.0) of variable aluminum stress in acid gleysol from Silver Valley for thirty days after sowing (DAS) (Experiment 2) relates to Figure 2.4a.

Source	df	MS	F-ratio
Block	3	0.002	1.34
Varieties	1	0.03	20.87**
pH	9	0.01	11.76**
Linear	1	0.12	95.50**
Quadratic	1	0.003	2.12
Cubic	1	0.004	2.97
Quartic	1	0.0003	0.25
Residual	5	0.005	4.39**
pH * Variety	9	0.002	1.24
Variety * Linear	1	0.01	5.94*
Variety * Quadratic	1	0.002	1.88
Variety * Cubic	1	0.001	0.38
Variety * Quartic	1	0.0001	0.06
Residual	5	0.001	0.59
Error	57	0.001	

** indicates significant difference at 1% level; * represents significant difference at 5% level.

Table 2.3. Repeated measures analysis of variance showing mean squares (MS) for root weights of Alikat (tolerant) and Katepwa (sensitive) growing under five levels (pH range from 4.4 to 5.5) of variable aluminum stress in acid gleysol from Silver Valley for thirty days after sowing (DAS) (Experiment 2) relates to Figure 2.4a.

Source	df	MS	F-ratio
Block	3	0.002	4.41*
Variety	1	0.030	49.47**
pH	4	0.004	0.74
Linear	1	0.160	284.97**
Quadratic	1	0.001	0.26
Cubic	1	4E-07	0
Residual	1	0.001	0.90
pH * Variety	4	0.010	0.12
Variety * Linear	1	0.0002	0.37
Variety * Quadratic	1	0.0003	0.37
Variety * Cubic	1	0.0001	0.14
Residual	1	0.0001	0.14
Error	33	0.001	

** indicates significant difference at 1% level; * represents significant difference at 5% level.

Root Growth

Root growth was affected in both tolerant and sensitive cultivars under aluminum toxic conditions. Fig. 2.4a. shows how the root weight of Alikat (tolerant) is nearly twice that for Katepwa (sensitive) at the soil pH value 4.4 during experiment 2. The data are in agreement with previous reports (Foy, 1984; Kerridge et al. 1971). This shows that there is a significant difference in the two genotypes due to the presence of the aluminum tolerance gene. In Katepwa plants it was observed that the roots were extremely stubby, reduced in length, lacked secondary and tertiary branching, were brownish in color and were brittle. All these agree with previous reports on aluminum-stressed roots (Alam, 1981; Clarkson, 1965; Fleming and Foy, 1968; Foy 1984; Foy et al. 1978; Kesser et al. 1975, 1977). There is no significant difference between the root dry weights of Alikat and Katepwa at a soil pH above 5.5. However, the results show that the root weights of the two genotypes converge at a soil pH above 6.0. The analysis of variance for root weight data (Table 2.1) shows that there is no significant interaction between variety and treatment and this is perhaps due to the small number of plants sampled during the experiment. This result is surprising because the two genotypes, Alikat (tolerant) and Katepwa (sensitive) are expected to perform similarly at soil pH beyond 5.5 when there is no aluminum stress. The best curve fit for root weight data was a linear curve

and the two slopes for the two genotypes were very significantly different (Table 2.2). However when only data for treatments up to a soil pH of 5.5 were analyzed it was seen that the two slopes of the two varieties did not differ significantly even though the means of the varieties were significantly different in the experimental model (Table 2.3). This lack of slope difference could be due to the small sample size and the considerable amount of 'background noise' which we can see in the size of the error bars in Fig. 2.4a.

Shoot Growth

Shoot growth difference was evident between the tolerant and the sensitive genotypes at very acid soil pH (e. g. 4.4 to 5.5), with growth of Alikat being superior, high standard errors for this trait. Beyond pH 5.5 there were not many noticeable differences in shoot growth. The analysis of variance for shoot dry weight data indicated that pH caused a significant difference in shoot dry weight and this agreed with previous reports (Fageria, 1982; Pavan and Bingham, 1982a, b; Alam, 1981). The cultivars were also different significantly but the interaction between the treatments and cultivars was not significant. The data agrees with the concept that root growth inhibition is a clearer indication than shoot growth inhibition as a response trait for aluminum toxicity (Clarkson, 1965; Foy, 1984). However symptoms of aluminum toxicity were noticed in the general poor stature of the aluminum sensitive plants of the genotpye Katepwa in comparison to those of the tolerant Alikat. The data points in Fig. 2.4b show that the Alikat and Katepwa response curves diverge above a soil pH 6.0. The divergence of shoot dry weight values is more than for root dry weight. This could be because they are not completely isogenic.

Total biomass yield

Total biomass was also differentially affected in the tolerant and the sensitive genotypes at very acid soil pH (4.4-5.7) (Alam, 1981; Fageria, 1982; Foy, 1984; Kerridge et al. 1971; Pavan and Bingham, 1982a). Above pH 5.7 there was not much difference in shoot growth. The analysis of variance for the total dry weight data indicated that pH caused a significant difference in biomass. The cultivars were also different significantly and the interaction between pH and cultivars was significant (Table 2.1). Total biomass of Alikat and Katepwa above pH 6.0 diverged slightly similar to shoot weight data and this could be explained by the fact that the two genotypes are not completely isogenic.

pH range for aluminum tolerance gene in Alikat-Katepwa near-isogenic pair

These experimental results how that there are significant differences in root and shoot growth due to the presence/absence of the aluminum tolerance gene at a soil pH range of 4.4 to 5.7. Differences in response between the tolerant and sensitive at (5.7-7.0) pH range cannot be easily explained because theoretically there is no aluminum toxicity stress at pH higher than 5.7. There could be the presence of other constraining factors on plant growth such as nutrient deficiencies in the soil medium, other metal toxicities and so forth. Other factors may be involved because of difference in the genetic basis, given that three backcrosses only give 87.5% genetic congruence. The experimenter is actually more interested in the response to pH range between 4.4 to 5.5 because this is the range where aluminum toxicity occurs. Thus the incorporation of the aluminum tolerance gene in the Alikat - Katepwa pair could be useful in developing an aluminum tolerant variety for Western Canada and other places where acidic high-aluminum soils prevail.

Experimental protocol

The control achieved in this experiment was good because the lime doses when added to the soil gave repeatable soil pH values. Fig 2.2 shows that the aluminum availability levels were much lower than those used in conventional nutrient culture experiments but these were successful in inducing significant differences in root growth in the two genotypes. Fig 2.2 also shows that effects due to free aluminum should not be expected in this soil type above pH 5.7. The data in experiment 1 showed that the growing of plants for a period of more than one month after sowing could increase the experimental error due to the fact that the roots grow excessively leading to a situation where aluminum stress is no longer a valid factor for comparison if roots extended into the non aluminum containing water bath. Draining the water from the trays at regular intervals would, as done in the experiment 2, be a good measure to ensure that no toxicity other than aluminum toxicity was present in the system.

2.4 Conclusions

The results indicate that due to the polymorphism of the aluminum tolerance gene in the near-isogenic pair there was a significant difference in the root, shoot, and total biomass when grown at pH (4.4-5.5). This difference in growth under aluminum toxic conditions in a narrow genetic background indicates for the first time the role of this particular tolerance gene source in a soil system, and its response to pH. The results very closely match those already demonstrated by other researchers in hydroponic systems, and confirm the pH 5.5 to 5.7 range as being that above which the aluminum tolerance source (gene) becomes ineffective.

Moreover, the response curves of the tolerant and sensitive near-isogenics at a wide range of toxicity indicates that growth is negatively affected in both genotypes at very low pH, but the tolerant isogenic grows faster than the sensitive. The tolerant is more responsive to lime dose and the root weight, and total biomass curves reach a plateau much more quickly than in the case of the sensitive. The intolerant isogenic Katepwa demonstrated much greater response to lowered pH (and associated Al increase) than did Alikat, for root mass, and total biomass. This would result in adaptive advantage in the early growth stages of plants with tolerant genotypes, at least partially due to an expected larger root proliferation for nutrient uptake, associated with the higher root weight. Despite high standard errors in the data, higher shoot weights were also demonstrated by Alikat compared to Katepwa under low pH, implying more potential for photosynthetic area. This aspect was studied in a separate experiment, reported in chapter 4.
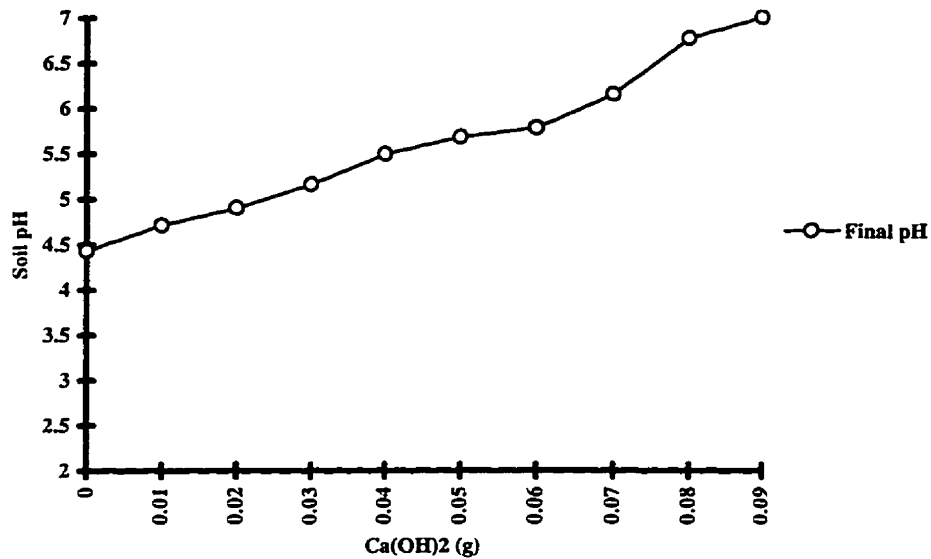
Fig. 2.1. Response of soil pH to the addition of Ca(OH)$_2$ to acid gleysol from Silver Valley (initial pH 4.4) measured using 1/1 ratio of soil to water w/w.
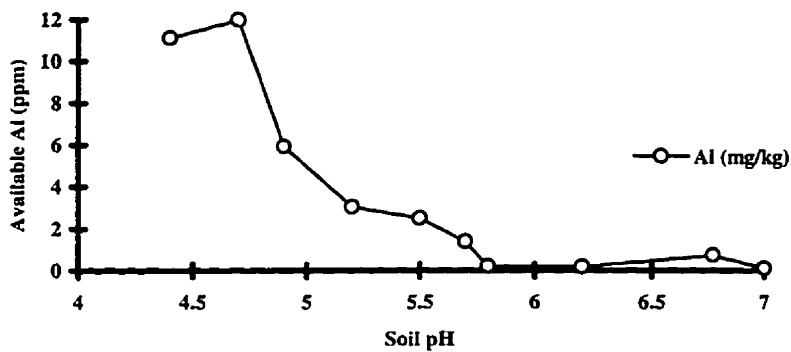

Fig. 2.2. Response of aluminum (mg/kg) availability in the soil solution to the addition of Ca(OH)$_2$ to acid gleysol from Silver Valley (initial pH 4.4) measured using 1/1 ratio of soil to water w/w.
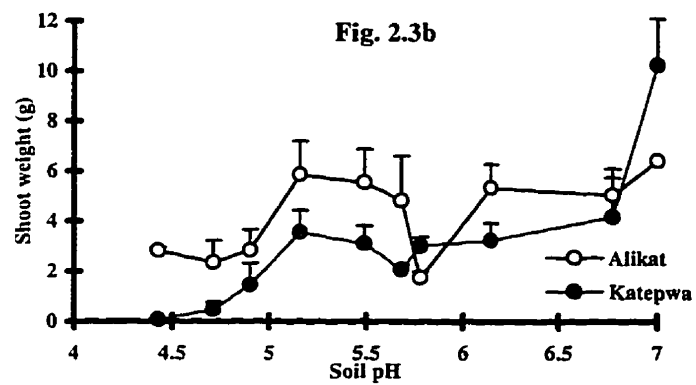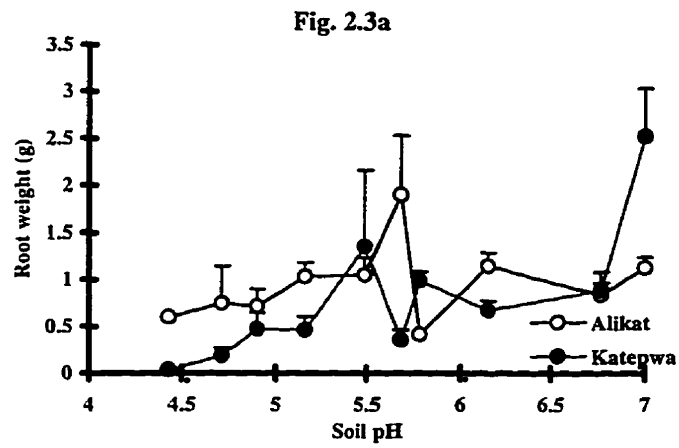
Fig. 2.3a

Fig. 2.3b

Fig. 2.3. Responses in root and shoot dry weights of Alikat (tolerant) and Katepwa (sensitive) plants grown in acid gleysol from Silver Valley for ninety days at various pH levels during experiment 1. Vertical bars represent standard errors.

Fig. 2.4a

Alikat
y = 0.0151x + 0.0466
R^2 = 0.162
Katepwa
y = 0.0477x - 0.1713
R^2 = 0.7554

O   Alikat
●   Katepwa
━━━Linear (Alikat)
━━━Linear (Katepwa)

Fig.2.4b

Alikat
y = 0.0179x + 0.2583
R^2 = 0.0349
Katepwa
y = 0.1691x - 0.6935
R^2 = 0.6644

O   Alikat
●   Katepwa
━━━Linear (Alikat)
━━━Linear (Katepwa)

Fig. 2.4c

Alikat
y = 0.0435x + 0.2589
R^2 = 0.0742
Katepwa
y = 0.217x - 0.862
R^2 = 0.6988

O   Alikat
●   Katepwa
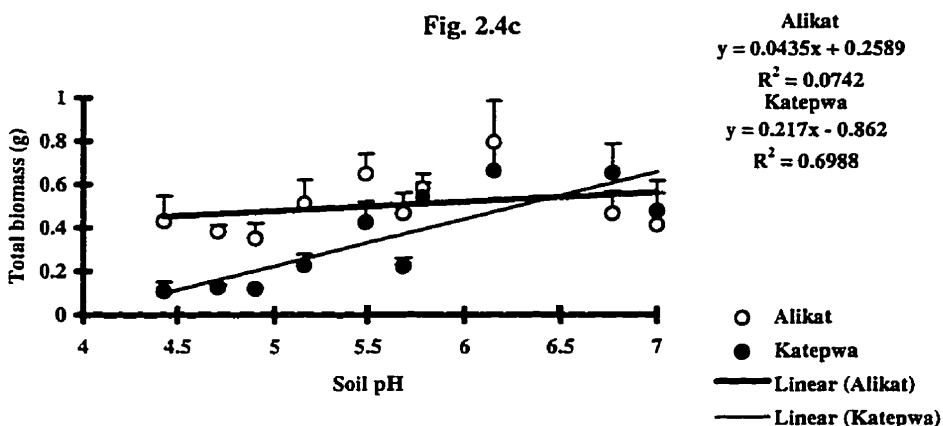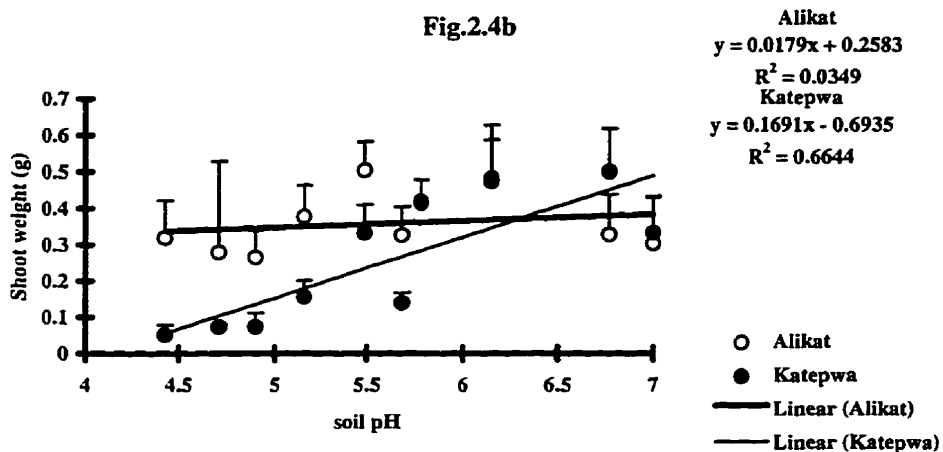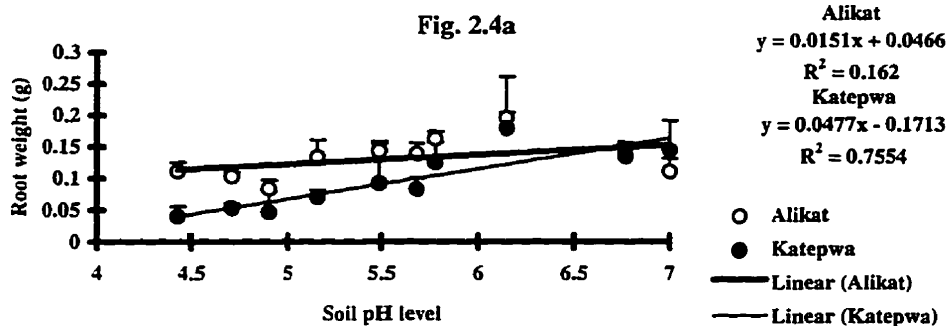━━━Linear (Alikat)
━━━Linear (Katepwa)

Fig. 2.4 Responses in root, shoot, and total dry weights in Alikat (tolerant) and Katepwa (sensitive) plants grown in acid gleysol from Silver Valley at various pH levels grown for thirty days during experiment 2. Vertical bars represent standard errors.

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Chapter III

Effect of long term aluminum toxicity on the yield of two near-isogenic lines differing for aluminum tolerance.

3.1 Introduction

Soil acidity has been identified as a major problem for growing crops for a good yield and the concern is growing among scientists in recent years who have made efforts to transfer Al tolerance in wheat lines (Briggs and Taylor, 1994; Carver et al. 1993; Fisher and Scott, 1987; Johnson et al. 1997). Soils with a low pH have high levels of exchangeable aluminum, iron and manganese which are phytotoxic, but aluminum seems to be the most toxic to plants growing on acid soils at a soil pH at or below 5.0 (Aniol, 1984; Camargo, 1981; Foy, 1988; Kerridge and Kronstad, 1968). Reduction of crop yield due to aluminum toxicity has been observed in several cases (Foy et al. 1978; Reich et al. 1981). Yield is a multigenic trait and it is a product of interaction between the genotype and the environment. Achievement of high yield in low-input environments and high-input environments often involve very different genes (Atlin and Frey, 1989). The amount of crop loss in Alberta or NE British Columbia due to soluble aluminum was studied for the first time by McKenzie (1973) and Penney (1973).

The idealized response curves of a tolerant and sensitive variety growing under aluminum stress have been proposed in a model by Scott and Fisher (1989). The model uses the data from LaFever et al. (1977). In this model, the tolerant cultivar is seen to outperform the sensitive cultivar at low pH (high aluminum stress), and increase its yield rapidly to a peak at a low lime rate of application under high pH condition. The sensitive cultivar however, is seen to respond more slowly to lime application, thus requiring more lime to reach the peak yield of the tolerant cultivar. The relevance of this model lies in the fact that the selection of tolerant germplasm based on yield in acid soil sites depends on the level of toxicity at the site. Thus it is important to study the response of tolerant and sensitive genotypes at various levels of toxicity. A similar model was described by Briggs et al. (1991) and Briggs and Taylor (1994), describing differential tolerance responses of plants grown in hydroponic systems. The latter paper describes Al dose response curves for Alikat and Katepwa, the same two cultivars studied in this thesis.

The combined use of breeding methods and agronomic practices is a viable solution for ameliorating the effects of aluminum toxicity. Increase in wheat yield by incorporating

aluminum tolerance and disease resistance has been reported in Zambia (Little, 1988). The use of near-isogenic lines for aluminum tolerance could be of great help in this respect. The presence of an aluminum tolerance gene in one of the genotypes in the near-isogenic pair is expected to produce a positive improvement in yield at a particular toxicity level. Moreover, differences in various yield components which contribute to the final product are also of interest to the breeder. Even though yield is a multigenic trait, the effect of the single aluminum tolerance gene in a near-isogenic pair for aluminum tolerance is an indicator of the reason why a particular genotype can produce a better yield than another genotype under the same level of aluminum stress. Moreover, a prolonged period of aluminum stress on growing plants is expected to have a very different effect than if the plants are exposed to stress for a short period as in the nutrient solution. As reviewed earlier, issues of adaptation to acute vs. chronic stress would be of relevance in studies where stress is prolonged (Section 1.2.4.2, chapter 1).

Alikat (tolerant) and Katepwa (intolerant) have been reported to differ in root growth, seed yield and total biomass yield in nutrient solutions while in the field they have demonstrated similar agronomic performance at neutral soil pH (Briggs, 1997 personal communication ; Briggs and Taylor, 1994). No study of yield performance involving near isogenic lines for aluminum tolerance under a wide range of soil pH has yet been reported.

The objective of this experiment was to study the effect of long-term aluminum toxicity in a greenhouse soil experiment on wheat plants in terms of the effect on the final seed yield, plant height, harvest index and total biomass of the near-isogenic plants of Alikat and Katepwa across a wide range of soil pH values (and associated aluminum toxicity). The response curves for yield of the two genotypes (tolerant and susceptible) grown at a range of pH were also of interest. It was expected that this would enable us to study the action of the aluminum tolerance gene on yield at these various pH levels, and to discover the critical pH range in which the difference between tolerant and susceptible plants would disappear. The use of the narrow genetic background (i.e., the near-isogenic lines for aluminum tolerance) would help in controlling the 'genotype' contribution to yield so that the association between tolerance and yield potential could be better understood.

3.2 Materials and methods

Plant material

Aluminum tolerant Alikat and sensitive Katepwa seeds were obtained from the University of Alberta, Experimental Research Station. Alikat (= Maringa*3/Katepwa) was

produced by backcrossing Maringa (Al tolerant), a Brazilian variety to Katepwa (Al sensitive). The method by which the seeds of Alikat were developed is described in chapter 2 under section 2.2.

Growth medium

The soil, an acidic, humic eluviated gleysol used in the experiment was brought from Silver Valley region in NW Alberta, Canada. The initial pH was 4.4, measured using 1/1 w/w soil to water ratio. The soil was treated with calcium hydroxide ($Ca(OH)_2$) and the soil pH response curve was prepared as previously described (Fig. 2.1). Aluminum concentration level varied with each of the ten soil pH levels (Fig. 2.2). A detailed description of the growth medium preparation has been given in chapter 2, section 2.2.

Plant culture

Plants were grown in half cut cylindrical tubes filled with acid soil containing lime treatments at various levels in the same way as has been described in chapter 2. Conditions for growth in these tubes and in the greenhouse chamber were also the same as described in chapter 2.

Experimental design and bioassay

The experimental design was a randomized complete block (2 x 10 factorial). There were ten soil pH levels indicating ten levels of aluminum stress, two cultivars, and three blocks as replications. The experimental unit was one plant in a soil tube. The plants were grown to maturity, after which the above ground part was harvested and subsequent measurements were made. All conditions under which the experiment was conducted and samples collected were the same as described in chapter 2. Plants were oven dried for dry weight measurement at 60^0C for 12 hours. Dry weights of shoot, seed weight, plant height were measured separately.

Data analysis

The data were analyzed using Analysis of Variance Procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Curve fitting response data to pH was done using repeated measures ANOVA (Petersen, 1985).

3.3 Results and discussion

The analysis of variance (Table 3.1) shows that total biomass, seed yields, and harvest index of the two cultivars were affected significantly by lime application whereas no significant effects on plant height were found. No genotype x pH interaction was found in any of the traits except for harvest index. The result is in agreement with previous reports on

yield under aluminum toxicity stress (Briggs and Taylor, 1994; Howeler, 1987; LaFever et al. 1977; Penney, 1973; McKenzie, 1973; Salinas and Sanchez, 1977; Sanchez, 1976; Spain, 1979; Ruiz-Torres et al. 1992). Seed yield of Alikat is almost four times that of Katepwa at pH 4.4 (Fig. 3.1) and appears constant up to pH 5.5. The seed yield of Katepwa rises slowly and yields the same as Alikat only at pH 5.5 (Table 3.3), i.e. after considerable lime application. Above pH 5.5, the trend in seed yield response curves is erratic. The regression equations of Alikat and Katepwa are significantly different over the soil pH range 4.4 to 5.5 (Table 3.3) but the R^2 values are quite small. Alikat seems to have an yield advantage over Katepwa even at high soil pH and this could be due to the ability to grow a better root system for the uptake of nutrients. Alikat seems to be less affected by changes in pH than Katepwa is. The repeated measures analysis of variance for seed yield indicates that the two genotypes differ at soil pH values 4.4 to 7.0 and that the best curve fit for the set of data was linear (Table 3.2). Table 3.2 shows that the two cultivars differed in seed yield over a wide range of soil pH (4.4-7.0) and the best-fit curves were found to be linear having similar slopes but with different intercepts (i.e., the genotype means). Table 3.3 however shows that Alikat and Katepwa seed yield response curves have different slopes with the pH range of 4.4 to 5.5. In field experiments it has been seen that while Alikat is similar in almost all agronomic characters, it differs from Katepwa in days to heading (Briggs and Taylor, 1994). This may contribute to Alikat's advantage in seed yield over Katepwa, even at neutral pH 7.0. Repeated measures analysis of variance of seed yield at the soil pH range of 4.4 to 5.5 however fails to fit a best-fit curve to the data(Table 3.3). This could be because of small sample size due to which the curve fitting test was less sensitive. Another explanation could be that plants growing up to maturity might face many other problems besides aluminum stress (Taylor and Foy, 1985). The response to lime application in both the genotypes in terms of seed yield seems to be higher for Katepwa. Alikat seems to be able to produce higher seed yield at lower lime doses. This could have an important bearing on breeding for yield increase in areas having the soil pH range 4.4 to 5.5.

Plant height was not affected by soil pH (aluminum availability in soil solution) in the two genotypes, and generally did not differ between the genotypes (Fig. 3.2, Table 3.1). This is not unusual because plant height is dependent on genotype. Moreover, Alikat and Katepwa are near-isogenic for aluminum toxicity and during the selection of Alikat plants (Katepwa x Maringa) the recurrent genotype was selected so that Alikat would be isophenotypic for height with Katepwa (Briggs and Taylor, 1994).

Harvest index of the two genotypes can be seen to be significantly different (Table 3.2, 3.3, Fig. 3.3). Harvest index is a ratio of the total seed weight to total biomass. There is also a significant pH x genotype interaction. However, the repeated measures ANOVA fails to find the best-fit curve. The reason could be the the two cultivars are isophenotypic for harvest index. A second reason could be the seed yield and total biomass of Katepwa are equally affected while in Alikat (Fig. 3.1, and 3.3). A third reason could be that the data is not described by a polynomial function - some other higher function describes the relationship.

Total biomass yield was significantly different for the two genotypes and varied among the ten lime treatments but there was no significant interaction between treatment and genotype (Table 3.1). The influence of aluminum stress on biomass production can depend on the effect of either the source limitation or the sink limitation or both. The data (Fig. 3.3) shows that total dry weight of Alikat is several times higher at pH 4.4 than that of Katepwa and continues to be significantly higher up to pH 5.5 when they become similar. This is similar to the results obtained with other aluminum tolerant genotypes in nutrient culture experiments (Moore et al. 1976; Spain, 1979) and wheat forage yield data under variable soil pH (E. G. Krenzer. Jr., unpublished data) (Carver and Ownby, 1995). The response of biomass above pH 5.5 in both Alikat and Katepwa shows that Alikat has an advantage over Katepwa even at neutral pH (7.0). The repeated measures ANOVA shows that the best fit curve for the two genotypes is linear (Table 3.2) but the slopes are not significantly different. The linear curves appear parallel to each other and hence there is no interaction between lime treatment and genotype under a pH range 4.4 to 7.0 (i. e. slopes of linear curves are not different). This means that genotype means differ significantly (shown in Fig. 3.3) and Alikat is less affected by pH of the soil than Katepwa. Alikat seems to produce more biomass than Katpwa even at high soil pH. However the repeated measures ANOVA in Table 3.3 where pH 4.4 to 5.5 data are analyzed does not show this trend, which could be due to the small sample size.

Table 3.1 ANOVA showing mean squares (MS) for total biomass, seed yield, plant height, and harvest index of Alikat (tolerant) and Katepwa (sensitive) plants grown to maturity in acid gleysol from Silver Valley .

Source	df	Biomass (MS)	Seed Yield (MS)	Plant Height (MS)	Harvest Index (MS)
Block	2	2.05	1.21**	145.87	0.03*
Soil pH	9	2.78*	0.48	260.16	0.01
Variety	1	24.78**	6.65**	582.10	0.08*
Soil pH*Variety	9	1.60	0.48	196.92	0.02*
Error	37	1.23	0.24	161.18	0.01

** indicates significant difference at 1% level; * represents significant at 5% level

Table 3.2. Mean squares of harvest index, biomass, and seed yield of Alikat and Katepwa grown to maturity in acid gleysol from Silver Valley limed to create a pH range 4.4-7.0, calculated using repeated measures analysis of variance procedure.

Source	df	Harvest Index (MS)	Biomass (MS)	Seed Yield (MS)
Block	2	0.01	1.49	0.72
Variety	1	0.07*	15.34*	4.40*
Soil pH	9	0.01	2.30	0.31
Linear	1	0	16.57*	2.17*
Quadratic	1	0	1.63	0.26
Cubic	1	0	0.01	0.03
Quartic	1	0.01	0.62	0.02
Residual	5	0.10*	1.83	0.32
Soil pH*Variety	9	0.01	1.11	0.26
Variety*Linear	1	0.01	0.72	0.10
Variety*Quadratic	1	0.02	3.90	1.36
Variety*Cubic	1	0.002	2.85	0.20
Variety*Quartic	1	0.02	0	0.13
Residual	5	0.06*	2.50	0.59
Error	38	0.01	2.38	0.40

* represents significant at 5 % level.

Table 3.3 Mean squares of harvest index, biomass and seed yield of Alikat and Katepwa plants grown to maturity in acid gleysol from Silver Valley limed to create a pH range 4.4-5.5, calculated using repeated measures analysis of variance.

Source	df	Harvest Index (MS)	Biomass (MS)	Seedyield (MS)
Block	2	0.01	0.01	0.09
Variety	1	0.09*	10.90	3.53*
Soil pH	4	0.02	0.96	0.15
Linear	1	0.001	0.92	0.16
Quadratic	1	0	2.04	0.33
Cubic	1	0.03	0.08	0.09
Residual	1	0.04	0.81	0
Soil pH*Variety	4	0.02	3.33	0.91*
Variety*Linear	1	0.06*	11.41	3.15
Variety*Quadratic	1	0.03	0.12	0.12
Variety*Cubic	1	0.01	0.85	0.04
Residual	1	0.01	0.95	0.34
Error	12	0.01	2.81	2.36

* represents significant at 5 % level.

3.4 Conclusions

When discussing 'aluminum toxicity' and its effect on yield, it is found that the toxicity mechanism is not a simple one involving only low pH, and toxicity stress, but may involve other inter-related factors such as poor root growth, reduced nutrient uptake, deficiency of Ca, Mg, and P and other such related problems (Taylor and Foy, 1985). The yield of tolerant and intolerant plants under aluminum stress is undoubtedly affected but in this study the tolerant genotype always performed better than the intolerant genotype in the range of pH (4.4 to 5.5) where aluminum in the soil solution can be toxic (evident in the low R^2 values in Fig. 3.1, 3.3, and 3.4). Nevertheless, the yield of Alikat (tolerant) was also negatively affected at the very acid pH of 4.4. It however, performed better than Katepwa (sensitive) at this level of toxicity and this might be due to better root growth (as seen in chapter 2) or due to greater photosynthetic area development (as seen in chapter 4) or both. The harvest index of the two genotypes were seen to be affected adversely at a low soil pH while at a neutral pH where there was no stress factor involved the trends became similar. This is expected because the genotypes are near-isogenic and are agronomically isophenotypic in non-stress soil (Briggs, 1997, personal communication). The response curve of the two genotypes, Alikat and Katepwa across a wide range of soil pH 4.4 to 7.0, showed a trend similar to the data of LaFever et al.(1977). It should be kept in mind that Alikat is a $BC*^3.F_2$ which means

the similarity with Katepwa in terms of genetic makeup is only about 87.5%. This study demonstrated that a single gene for aluminum tolerance can produce a tolerant genotype which performs better than the intolerant at a range of pH from 4.4 to 5.5. It requires less lime application to reach the highest yield potential at pH 5.5, at which pH most of the effects of toxicity disappeared. Thus breeding to incorporate the aluminum tolerance gene into spring wheat varieties which are aluminum-sensitive can increase yields significantly with low doses of lime application. Conversely, the ability of the tolerance source to maintain yield potential is greater at lower pH, compared to the intolerant one.

Fig. 3.1. Seed yield under variable soil pH

$y = 0.2332x + 0.312$
$R^2 = 0.2815$

$y = 0.3181x - 0.8525$
$R^2 = 0.4081$
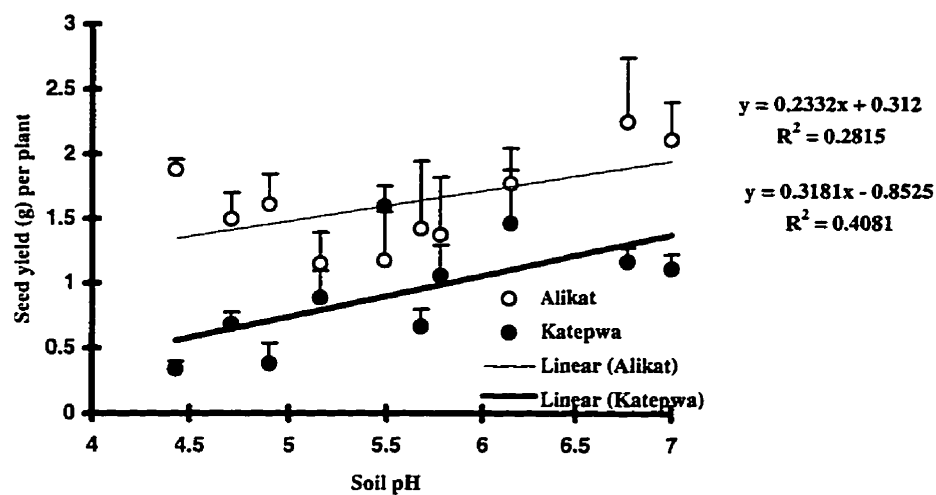
Fig. 3.2. Plant height under variable soil pH


$y = 2.2658x + 51.595$
$R^2 = 0.169$

$y = 4.5889x + 33.987$
$R^2 = 0.2023$
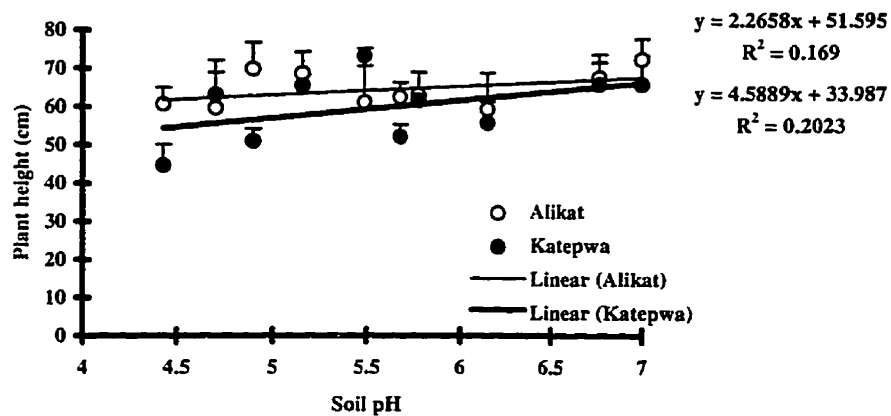
Fig. 3.1 and 3.2. Seed yield and plant height per plant under greenhouse conditions for aluminum tolerant (Alikat) andsensitive (Katepwa) isogenic lines grown in acid soil limed to different soil pH levels.

Fig. 3.3. Plant biomass under variable soil pH

$y = 0.6431x + 0.5188$
$R^2 = 0.3672$

$y = 0.7635x - 1.4767$
$R^2 = 0.6219$

—O—Alikat
—●—Katepwa
——Linear (Alikat)
——Linear (Katepwa)
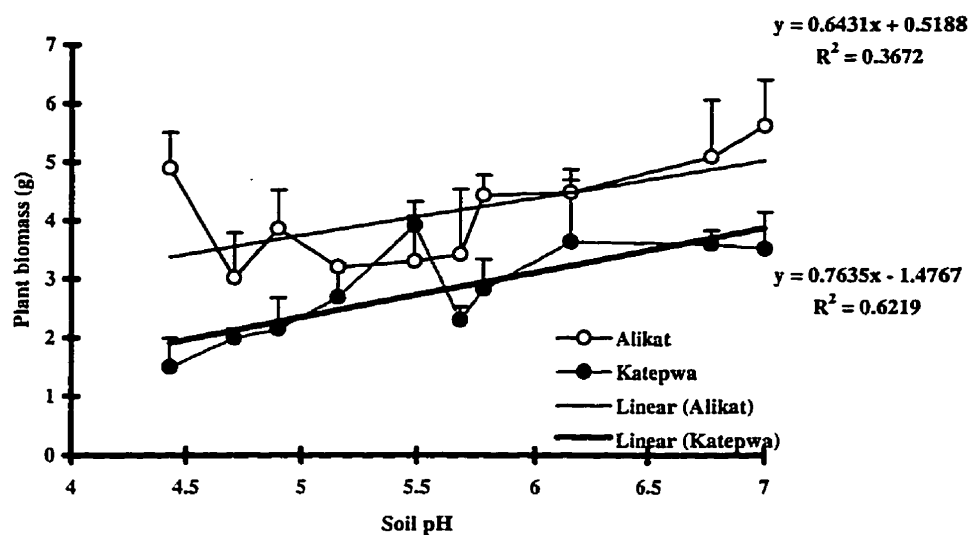
Fig. 3.4. Harvest index at variable pH

$y = -0.0164x + 0.4867$
$R^2 = 0.0487$

$y = 0.0287x + 0.1571$
$R^2 = 0.1395$

—O—Alikat
—●—Katepwa
——Linear (Alikat)
——Linear (Katepwa)
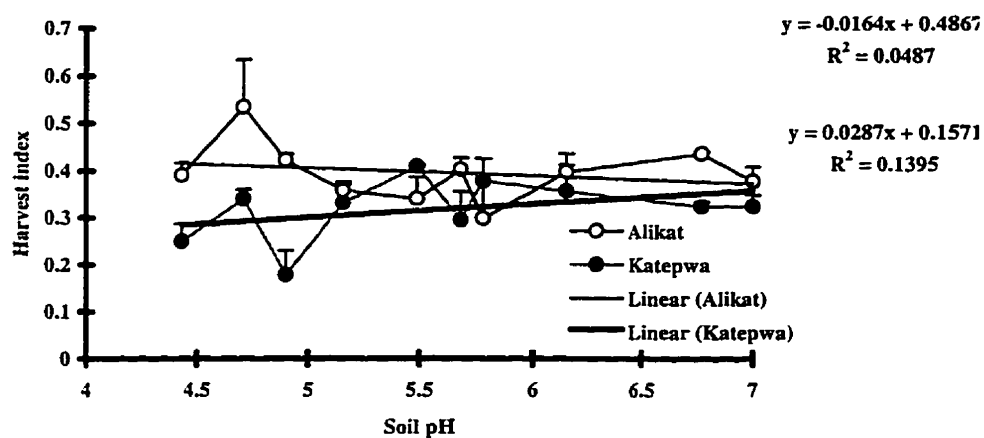
Fig. 3.3 and 3.4. Plant biomass and harvest index per plant under greenhouse conditions for aluminum tolerant (Alikat) and sensitive (Katepwa) isogenic lines grown in an acid gleysol limed to different soil pH levels.

Fig. 3. 5. Seed yield at variable soil pH
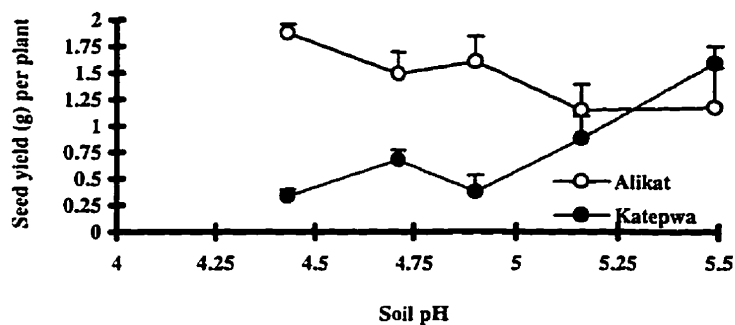
Fig. 3.6. Plant biomass at variable soil pH
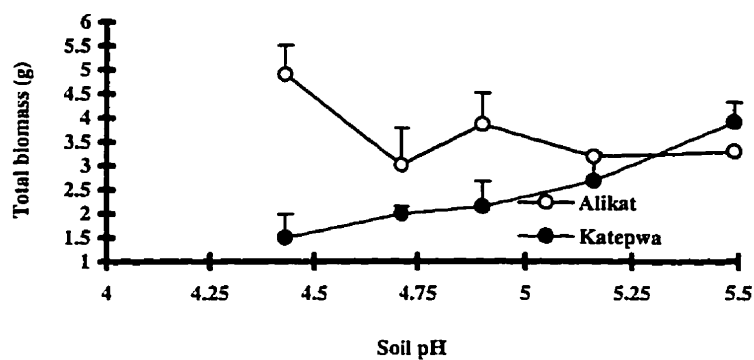
Fig. 3.7. Harvest index at variable soil pH
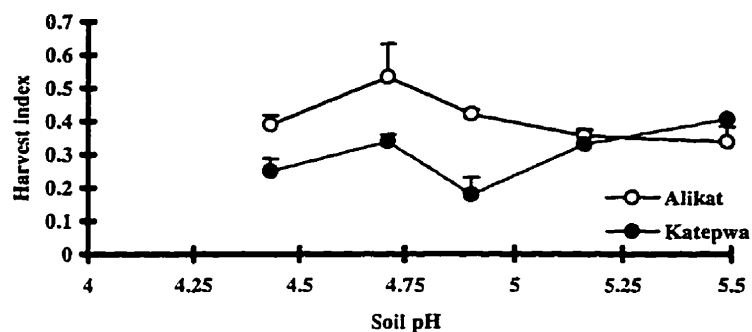
Fig. 3.5, 3.6, and 3.7. Seed yield, plant biomass and harvest index per plant under greenhouse conditions for aluminum tolerant (Alikat) and sensitive (Katepwa) isogenic lines grown in acid gleysol limed to different soil pH levels.

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Chapter IV

Leaf growth during the early seedling stage in aluminum tolerant/intolerant near-isogenic genotypes under variable soil pH conditions with varying levels of aluminum.

4.1 Introduction

Soil acidity is a growth limiting factor in soils all over the world (Foy, 1988). Aluminum toxicity is one of the most important reasons for crop loss in acid soils (Foy, 1988). Aluminum toxicity causes reduction in root growth and a decrease in final yield of the crop. Besides the stunting of root growth and damage to root tip cells, basic physiological processes such as the uptake of water and minerals are affected. Plants become more susceptible to drought in aluminum toxic soils due to restricted root development (Foy and Fleming, 1978). Thus there is an additional stress imposed on the plants due to drought or a scarcity of water. Aluminum toxicity in the topsoil layer can be remedied by lime applications to the top layer of soil although the subsoil aluminum is still a problem to plant roots growing in that zone. Due to this artificial drought stress in the presence of aluminum, effects such as decrease in leaf water potential, photosynthesis, transpiration rate and chlorophyll concentration have been observed in wheat (*Triticum aestivum* L.) (Kaufmann and Gardner, 1978; Ohki, 1986). A decrease in vegetative growth of the susceptible barley (*Hordeum vulgare* L.) cultivar Kearney was seen as a result of aluminum stress (Krizek and Foy, 1988). In sunflower (*Helianthus annus* L.) similar drought stress effects were observed to be caused by aluminum toxicity (Krizek et al. 1988). Goldman et al. (1989) concluded that there might be an interaction between drought and aluminum stress. Water deficit in the plant caused by stunted root growth might lead to reduction in leaf expansion and leaf area development, although direct evidence of this interaction is not available.

Leaf area plays an important role in determining the amount of water use and carbon uptake and therefore in the long run it affects the potential productivity of the plant (Levitt, 1972). Leaf area is decreased due to drought stress and this takes place due to reduction in leaf water status (Boyer, 1985). Reduction in leaf area has been observed in water-stressed cotton plants (Rosenthal et al. 1987) where it has been shown that individual leaf area and leaf cells are smaller (Cutler and Rains, 1977). Reduction in the total leaf area takes place for two reasons: (i) decrease in the total number of leaves or number of leaves in each branch (Steinberg et al. 1990; Ney et al. 1994; Muchow et al. 1986), and (ii) decrease in the number

of branches or tillers (Norris, 1982; Davidson and Chevalier, 1987). The Haun scale (Haun, 1973) has been used to describe the stage of vegetative development in wheat (Lafond and Baker, 1986), durum wheat (Bauer et al. 1984), winter wheat (Krenzer et al. 1991) and wild oat (Cudney et al. 1989). The Haun scale quantifies the developmental stage of the plant by expressing the number of fully expanded leaves as an integer and the ratio of the length of the youngest leaf to the length of the last fully expanded leaf as a decimal fraction (Haun, 1973). The Haun scale has been stated to be the most sensitive among other scales to daily responses of plant morphology to several environmental factors (Bauer et al. 1984). It has been observed that the mainstem leaf stage is affected by water stress (Krenzer et al. 1991). To achieve a good yield potential early emergence and development of the leaves and the canopy is necessary (Gan and Stoebbe, 1996; Gan et al. 1992). The response of the plant root to toxic aluminum has been found to take place as early as 30 minutes after exposure in nutrient culture (Zhang et al. 1994) but these investigations on early seedling growth and development have not been repeated in a soil system. Moreover, no study of total leaf area development under aluminum-toxic conditions over a temporal scale has yet been reported.

The objective of this study was to investigate the responses during the early seedling growth in the number of leaves, total leaf area, Haun stage of leaf development, root and shoot weight increase, increase in longest leaf length, and response in the length and width of the first leaf in two near-isogenic lines for aluminum tolerance grown under variable levels of aluminum stress.

(i) Experiment 1 was carried out with the objective to study whether Haun scale values, root weight, shoot weight, and longest leaf length were affected significantly when the two cultivars were grown in unlimed (pH 4.4), limed (pH 4.9, 5.5), and neutral soil pH 7.0. The aluminum tolerance gene was expected to act in the pH range of 4.4 to 5.5 but it would also be interesting to study the response at pH 7.0 where there was no aluminum toxicity.

(ii) Experiment 2 was carried out with the objective of studying Haun scale values, total leaf area, total number of leaves, length and width of the first leaf at pH levels 4.4, 4.9, 5.5, and 7.0 to obtain insight into the relative development of the total leaf area in the early developmental stage. Experiment 2 was a further follow up of experiment 1 using more replications and measuring different traits.

4.2 Materials and methods

Plant material

The seeds of two near-isogenic spring wheat (*Triticum aestivum* L.) lines for aluminum tolerance, Alikat (tolerant) and Katepwa (susceptible) were grown in the greenhouse in Silver Valley soil. The seed was obtained from the University of Alberta, Experimental Research Station. Alikat is a BC_3F_2 produced by back-crossing Brazilian cultivar Maringa (Al-tolerant) to Katepwa (Al-susceptible) (Zale and Briggs, 1988) and this has been described in detail in chapter 2.

Growth medium

Plants were grown in Silver Valley Soil, an acidic gleysol which had a pH (determined using a 1:1 soil to water ratio) of 4.4 which was considered highly acidic for growth of wheat. The soil was treated by adding varying levels of calcium hydroxide ($Ca(OH)_2$) to raise the soil pH (pH levels 4.4, 4.9, 5.5, 7.0) (Fig. 2.1) and consequently to change the level of available aluminum in the soil solution (Fig, 2.2). These levels of pH were chosen because they represented the range of soil pH (4.4-5.5) where aluminum was considered most phytotoxic and they also included intermediate and neutral pH (4.9, 7.0). Growth medium conditions are described in chapter 2 in section 2.2.

Plant culture

Seeds were sown to a depth of 1.5 cm below the soil surface in pots 6 cm in diameter. There were three plants per pot. Plants were grown in the greenhouse. The average temperature in the greenhouse was maintained at 21 °C and an average relative humidity of 81%. The controller vent temperature for the greenhouse chamber was set at 89°F (32°C). Photoperiod hours for both experiment 1 and 2 were 16. Sunlight hours of 16 hours were supplemented by halogen lamps described in chapter 2, in section 2.2. Plants within a block were randomized regularly to reduce the effects of bench position on pots within a block. Pots were irrigated regularly so that the plants would not be subjected to water stress. The soil was always watered to maintain field capacity.

Experimental design

The experimental design in both experiments was a split-block experiment (Steele and Torrie, 1986). The model used was a mixed model with block as a random effect and variety (genotype), pH, and time of harvest as fixed effects. In experiment 1 there were three replications as blocks and within each block there were three pots per treatment. In experiment 2 there were four replications as blocks and three plants sown per pot which was the experimental unit in both the experiments.

Data and Statistical analysis

All plants emerged 5 days after planting. Plants were then harvested at 1. 3. 7. and 15 days after emergence (DAE). Plants were placed in airtight plastic bags immediately after harvest of experiment 2 samples and placed on ice so that there would be no change in dimensions due to drying. Root weight, shoot weight, longest leaf length in experiment 1. and total leaf area, length and width of first leaf were measured and Haun scale values in experiment 2 were calculated. Total leaf area was measured using a leaf area meter. Individual leaves were taken out carefully from the stem and their length measured from the base of the ligule. The leaf width was the width at the part where the leaf was the broadest. Plant samples were oven dried at $60^{o}C$ for 12 hours for dry weight measurement after the longest leaf length and Haun scale values had been recorded. Statistical analyses of data were done for both experiment 1 and 2 using PROC ANOVA procedure of SAS (1987). All main effects with the exception of replications or blocks were considered as fixed effects. Block x pH x variety was considered as Error 1 and pH and variety were tested against this. Time. time x pH. time x variety. time x pH x variety were tested against block x time x pH x variety which was Error 2 (Steele and Torrie. 1986).

Table 4.1. Mean squares (MS) of Haun scale values, longest leaf length and root weight for near-isogenic wheat cultivars for aluminum tolerance grown under variable soil pH in acid gleysol from Silver valley (experiment 1).

Effect	df	Haun scale MS	Longest leaf MS	Root weight MS
Block	2	0.076	32.952*	0.00004
pH	3	0.503*	143.93*	0.0001*
Variety (V)	1	0.999*	338.506*	0.00005
pH* V	3	0.026	31.911*	0.00007
Error I	14	0.075	6.185	0.0002
Time (T)	3	20.282*	2313.512*	0.0009*
T * Block	6	0.119	6.529	0.00003
T * pH	9	0.082	10.621	0.00044
T * Variety	3	0.043	27.909*	0.00003
T * pH* V	9	0.132	15.552*	0.00006*
Error II	42	0.093		0.00002

* indicates significant difference at 5% level.

Table 4.2. Mean squares (MS) of Haun scale values, number of leaves, total leaf area, leaf width and leaf length of the first leaf of spring wheat near-isogenics for aluminum tolerance grown at variable soil pH levels in acid gleysol from Silver Valley (experiment2).

Effect	df	Haun scale Value MS	Number of leaves MS	Total leaf area MS	Leaf width MS	Leaf length MS
Block	3	0.0320	0.2440*	0.2015	0.0002	4.4290
pH	3	0.8390*	0.5070*	75.7510*	0.0480*	37.9840*
Variety (V)	1	0.4200*	0.0230	5.8570*	0.0480*	67.5200*
pH* V	3	0.0580	0.0460	2.0670*	0.0170*	11.0750*
Error I	21	0.0790*	0.1250	0.2570	0.0007*	1.7340
Time (T)	3	29.7530*	19.8070*	486.4900*	0.0390*	272.390*
T * Block	9	0.0140	0.0540	0.3410	0.0010*	3.7880
T * pH	9	0.4670*	0.3390*	55.3590*	0.0030*	17.5400*
T * Variety	3	0.2970*	0.0910	1.2380*	0.0050*	6.3070
T * pH*V	9	0.2050*	0.2570*	1.1910*	0.0006*	11.6020*
Error II	63	0.0320	0.0820	0.2890	0.0003	2.4620

* indicates significant difference at 5% level.

4.3 Results and discussion

In experiment 1 data ANOVA (Table 4.1), it was observed that for Haun Scale values. pH, variety, and time of harvest effects were significant while pH x variety, time x pH, time x variety, and time x pH x variety effects were not significant. Effects on longest leaf length of pH, variety, time were significant as well as time x pH x variety interaction (table 4.1). Root weight data ANOVA showed that effects of pH and time and the time x pH x variety interaction were significant.

In the experiment 2, ANOVA (Table 4.2), there was a significant effect on Haun scale values due to pH, variety (genotype), and time of harvest. The time x pH interaction, time x variety and time x pH x variety interaction were also significant. Total number of leaves were significantly affected by pH, time, time x pH and pH x time x variety. In case of the total leaf

area, the effects of pH, variety, and pH x variety interaction were significant. Time, time x variety and time x pH x variety effects were also significant. There were significant effects among pH, variety and pH x variety interaction for leaf length of the first leaf. Time, time x pH, time x variety and time x pH x variety were all significant for leaf length. Leaf width differences due to pH, variety, pH x variety, time, time x pH, and time x pH x variety were all significant.

Haun Scale value, root weight, shoot weight, longest leaf length

Stored chemical energy in the cotyledon supplies nourishment to the embryo until the time when the green leaves are developed enough to produce photosynthate for the growth and metabolism of the plant. The germination pattern of the seeds in acid soil was not studied since all measurements were taken after the emergence of the first leaf. The seedlings of both the genotypes were observed to emerge at approximately the same time i.e.. five to six days after planting, provided there was a constant water supply to the soil. The seeds were not pre-germinated on moist filter paper because it was thought that this would not simulate the actual acid soil conditions encountered by the seeds in the field. The acid soil was thought to be a factor which could affect the rate of emergence of the embryo and its subsequent growth. although data were not collected to test this concept.

Haun scale values for Alikat (tolerant) and Katepwa (susceptible) Fig. 4.1(a-d) showed distinctly different trends at pH 4.4 and 7.0. At soil pH 4.4, Alikat had an advantage over Katepwa due to the greater rate of increase of Haun Scale value over time than Katepwa. This probably reflects the advantage Alikat has over Katepwa in root growth which was seen in the results of experiment 3 (Fig. 4.3a-b). The early establishment of the seedling takes place at first by using nutrients which are stored in the seed endosperm. As the nutrient reserves become depleted the growing embryo switches from heterotrophic to autotrophic mode of nutrition. The establishment of the first emerging roots in the soil is crucial in this respect because they help in anchorage of the seedling in the soil and in drawing nutrients form the soil and this has an effect on early growth of the seedling and its subsequent growth. The establishment of the shoot and root system of the growing seedling is accomplished with the help of the stored chemical energy in the seed endosperm and this process is largely influenced by soil environmental factors. At pH 7.0 the development of the two near-isogenic lines closely resembled each other because there was no aluminum toxicity at that soil pH. At pH 4.9 the Al-tolerant and Al-sensitive showed differences in rates of increase in the Haun scale value.

However, at 5.5 pH where aluminum toxicity is theoretically supposed to have become almost negligible, the Haun scale values of Alikat plants were still higher different than that of plants of variety Katepwa. Haun scale values are easily influenced by environmental stress (Bauer et al. 1984) and is well suited to descibe stages of wheat growth (LaFond and Baker, 1986). Differences in Alikat and Katepwa Haun scale values therefore probabaly reflect aluminum toxicity stress.

Longest leaf length curves of Alikat and Katepwa Fig. 4.2(a-d) show a trend very similar to that of shoot weight. There are increasing differences in longest leaf length with time between the tolerant and susceptible plants at 4.4, and 4.9 pH. These differences decrease at pH 5.5, and at pH 7.0 the two genotypes have very similar longest leaf length which indicates their near-isogenic background. These results parallel the shoot dry weight trends in Fig. 4.4(a-d) seen in experiment 1, as expected.

Root weights of Alikat and Katepwa Fig. 4.3(a-d) at pH 4.4 differed greatly at 15 days after emergence of the seedling. This is similar to previous results obtained in the Alikat-Katepwa near-isogenic system (Briggs and Taylor. 1994). The total root weight in Katepwa started to decline 7 days after emergence in contrast to that of Alikat which had an increasing trend. This could be due to the increasing damage caused on the root surface by toxic aluminum species. This probably indicates that the rate of root weight increase in Alikat is much higher than that of Katepwa at 4.4 pH. Similar results were observed in data from Taylor and Foy (1985), Briggs and Taylor (1994) and Briggs et al. (1992). The presence of an aluminum tolerance gene in Alikat helps the roots to extend downwards and grow in the soil at pH 4.4. Root weight increase trends of both Alikat and Katepwa were similar at pH 4.9. 5.5 and 7.0.

Shoot weight increases Fig. 4.4(a-d) show trends similar to root weight increases. However differences increased progressively from three days onward between Alikat and Katepwa at pH 4.4. At pH 4.4 the shoot weight curve for Alikat seems to be still rising whereas the curve for Katepwa seems to rising upwards at a lower rate than Alikat. This slower rate of shoot growth in Katepwa plants is probably because their roots are severely affected by Al toxicity and are unable to draw nutrients in sufficient quantities for the growth of the plant. The differences in shoot weight decrease gradually with time when the plants are grown in soil pH level of 4.9. 5.5. until at pH 7.0 Alikat and Katepwa appear to have similar shoot growth rates. At pH 7.0. after 15 days after emergence, Katepwa and Alikat seem to have a similar shoot weights. There

are reports that shoot growth responses are not always well correlated with root growth data (Zale, 1987).

Total leaf area, total leaf number, length and width of first leaf

The total leaf area and rate of photosynthesis of a plant are factors which control yield. Leaf area is sometimes more important than the rate of photosynthetic activity in affecting the yield of a crop (Gifford and Evans, 1981). Leaf area development takes place due to the expansion of cells by cell division and cell enlargement. In monocot leaves dividing cells occur at the leaf base and as cells divide they elongate the cells above them; thus leading to leaf development. The expansion of a leaf takes place as a result of turgor pressure on the cell wall which causes the wall to expand. The expansion due to turgor pressure is however very sensitive to environmental stress, including water scarcity (Hsiao, 1973).The interaction of genetic factors of the plant with the environment influences the expansion of a leaf and its final size (Hinckley et al., 1989).

The total leaf area curves of Alikat (tolerant) and Katepwa (susceptible) Fig. 4.5 (a-d) show that at pH 4.4 the tolerant line has a greater leaf area than the susceptible one. There is a large difference in total leaf area from a very early stage i.e., 3 days after emergence. The total leaf area for both Alikat and Katepwa at pH 4.4 are lower than at pH 4.9, 5.5 or 7.0. At pH 4.9 the total leaf areas of Alikat and Katepwa are the same. The standard errors at pH 4.9 for both Alikat and Katepwa are slightly larger than those at pH 4.4 and for this reason significant differences between the leaf areas of the tolerant and susceptible types are not clearly observed. At pH 5.5 there is also an increasing difference in total leaf area after 7 days after emergence even though the aluminum toxicity level is considered negligible at this pH. Therefore, it might be concluded that the near-isogenic lines may again be non-isogenic for other genes which control total leaf area. At pH 7.0, however, the two curves (tolerant and susceptible) merge and the total leaf areas of each line are very similar. The total leaf area curve shows an increasing trend at pH 4.9, 5.5 and 7.0 for both Alikat and Katepwa whereas at pH 4.4 the curves for both the genotypes seem to be nearing a plateau where the rate of total leaf area increase decreases. The leaf area is a result of several factors, number of total leaves on the stem being one. In previous reports leaf area has been seen to be affected by water stress (Boyer, 1985; Rosenthal et al. 1987) but there are no prior reports describing total leaf area reduction under aluminum stress.

The total number of leaves is also expressed as the Haun Scale value (Haun 1973). The total number of leaves can be influenced by the environment and this could lead to reduction in total leaf area (Steinberg et al. 1990). Generally the number of leaves on a stem is controlled genetically whereas the number of tillers or branches is influenced by environmental stress such as drought. This has been observed in forage grasses (Norris, 1982), in wheat (Davidson and Chevalier, 1987), and in peach trees (Steinberg et a. 1990). In experiment 2, data were recorded regarding the number of leaves Fig. 4.6 (a-d). No differences were found in the number of leaves of Alikat and Katepwa at pH 4.4. At pH 4.9 the curve shows there is a small difference in number of leaves after 7 days after emergence, but the differences were not significant. At pH 5.5 and 7.0 the number of leaves of Alikat and Katepwa are nearly the same. The number of leaves at both pH 5.5 and 7.0 levels continue in an increasing trend upwards whereas at pH 4.4 and 4.9 the number of leaves of Alikat seems to plateau while Katepwa appears to be trending towards a decline at pH 4.9. In general both Alikat and Katepwa appear to develop less new leaves after an initial period of increase up to 7 days after emergence. The standard errors are quite large for both Alikat and Katepwa at 15 days after emergence and this could be due to the small size of the sample. However it appears that leaf production of Alikat and Katepwa slows as the toxicity effect accumulates at pH 4.4 and 4.9. The number of leaves on the main stem is much lower than the field values observed in non-stress soil. Apparently, there seems to be a plateauing effect in the total number of leaves after four to six days after emergence. This is in contradiction to what is noticed in the field where the soil is neutral in pH.

The leaf length of the first leaf Fig.4.7 (a-d) at pH 4.4 differed largely for Alikat and Katepwa (3 and 7 days after emergence). This difference in leaf length was however decreased at 15 days after emergence. Due to the initial difference in leaf length which leads to difference in total leaf area, there is probably a cumulative effect on the photosynthetic leaf area and this can be responsible for later loss in yield. At pH 7.0 the two genotypes behaved very similarly in terms of leaf length of the first leaf. At pH 4.9 the difference in first leaf length increased after 7 days after emergence. This might be due to the fact that there were large standard errors of mean in the data points due to the small sample size. At pH 5.9 the difference in leaf length was considerable at 7 days after emergence but the difference was less at 15 days after emergence. The curves show a trend towards the increase of leaf length becoming constant after 15 days after emergence at pH 4.4 and 4.9, which are pH levels where toxic aluminum

effects occur. At pH 5.5 and 7.0 the curves show an increasing trend because the Al toxicity levels are almost negligible. Individual leaf area and leaf size have been reported to be reduced due to water stress in cotton (Rosenthal et al. 1987) but these are the first reports about effects of aluminum tolerance gene(s) on leaf size and area reduction under aluminum toxicity stress.

Successive leaves formed on the stem tend to increase in width. The leaf width is not thought to be influenced by environmental factors as much as is leaf length. The final leaf length is a reflection of the interaction between the genetic factors of leaf length and environmental factors which affect basic physiological processes controlling leaf expansion. such as turgor pressure against the cell wall (Hinckley et al., 1989). The graphs showing leaf width of the first leaf at consecutive harvest times for Alikat and Katepwa Fig.4.8(a-d) show that the leaf width values do not show a great deal of variation over time at different soil pH levels. Leaf widths of the first leaf at pH 5.5 and 7.0 are almost similar to each other, while at pH 4.4 and 4.9 the leaf widths of Alikat and Katepwa at early harvest times (3 and 7 days after emergence) differ noticeably. At 15 days after emergence at pH 4.4 these differences are reduced. At pH 4.9, however the differences in leaf width of the first leaf are wide at 3 days after emergence, are narrowed down at 7 days after emergence and grow wider at 15 days after emergence.

4.4 Conclusion

Early seedling growth is crucial for the plant's potential productivity (TeKrony and Egli. 1991). This is more important in small-seeded crop species such as cereals rather than in large-seeded crops such as cotton, corn and soybean. Seedling vigor in the early growth stage helps the plant to intercept more light energy and access more nutrients if there is a rapid increase in shoot and root growth. A plant having a greater shoot growth can intercept more energy and assimilate more carbon and can sustain more biomass in the subsequent period. Thus there is a "compound interest relationship" between current growth and future yield. With adequate agronomic inputs seedling vigor might not be a very vital component contributing to yield. but in stressed environments or in resource-poor environments seedling vigor becomes even more important.

The Haun scale values of Alikat (Al-tolerant) and Katepwa (Al- susceptible) at pH 4.4 compared to pH 7.0 at very early seedling stages of 3, 7 days after emergence show that Alikat has a higher growth rate than Katepwa. This could be due to its greater ability to tolerate aluminum in the growth medium during a very early stage and to extend more roots into the

soil. From the leaf length and width data of the first leaf it might be said that the leaf length is more important in affecting total leaf area than is leaf width. The initial difference in leaf area during 15 days after emergence is probably crucial, giving rise to a difference in total leaf area between Alikat and Katepwa under aluminum stress and this leads to a reduction in overall growth and plant yield potential. Haun Scale values and total leaf area data account for the differences in root weight and shoot weight observed in the two genotypes at low pH (4.4), where there is a high level of aluminum stress. The results indicate that there is an effect on Haun scale values (a measure of developmental rate), root weight, shoot weight growth rates, longest leaf length, total leaf area, total number of leaves, and leaf length and width of the first leaf produced due to aluminum stress. The aluminum tolerance gene obviously produces differences in plants growing at high stress conditions while at a neutral soil pH tolerant and susceptible plants are indistinguishable. Root growth and leaf area are clearly seen to be affected by toxicity of aluminum. Therefore it can be concluded that by introduction of this genetic source of tolerance seedlings can be made to grow faster at high stress conditions. This is the first report of aluminum toxicity influencing both root and leaf growth which has important agronomic consequences.

Fig. 4.1a. Haun's Leaf scale at pH 4.4
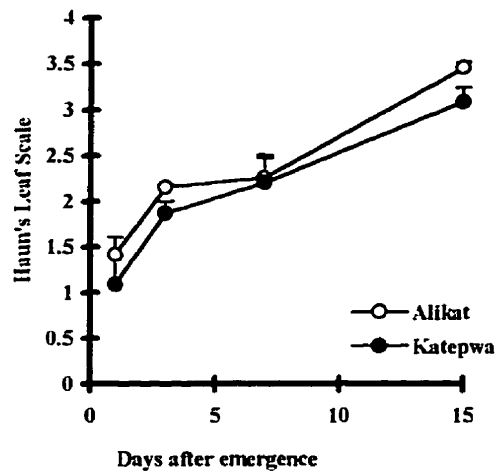
Fig. 4.1b. Haun's Leaf scale at pH 4.9
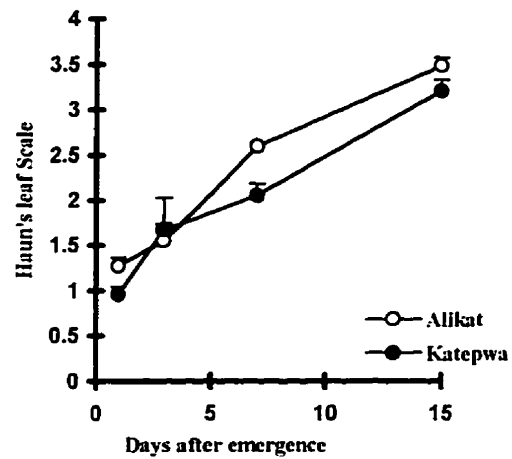
Fig. 4.1c. Haun's Leaf scale at pH 5.5
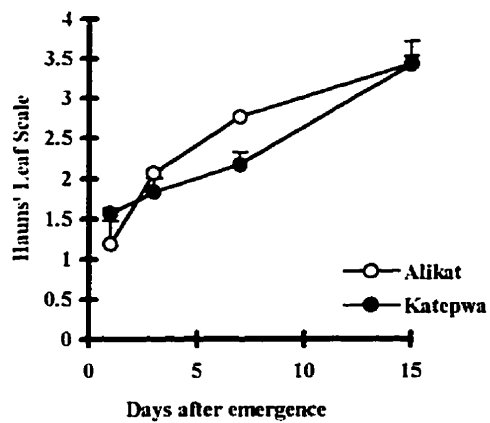
Fig. 4.1d. Haun's Leaf scale at pH 7.0
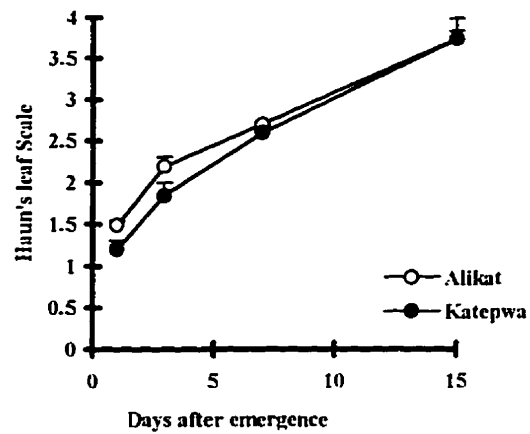
Fig. 4.1. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0
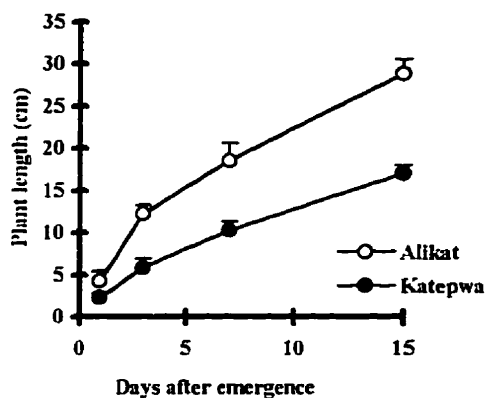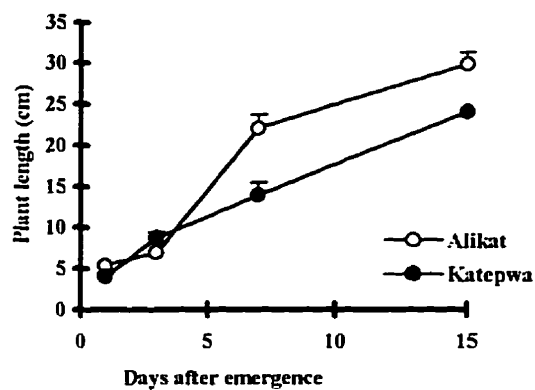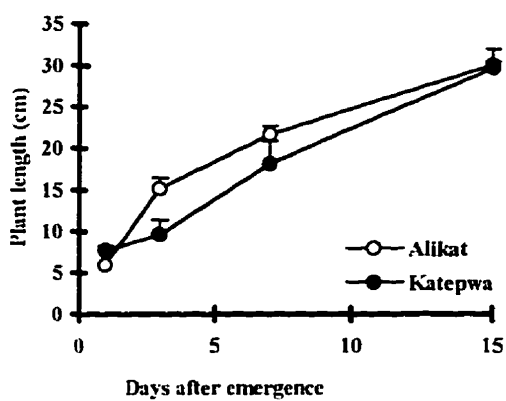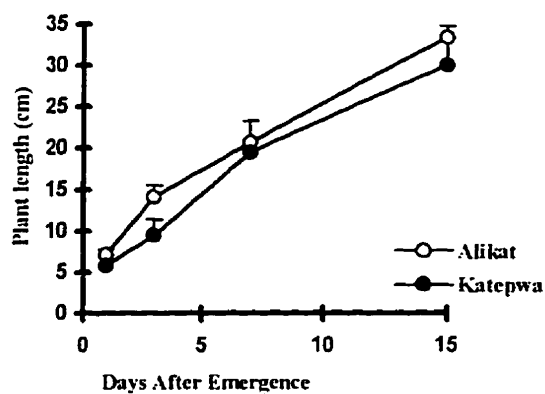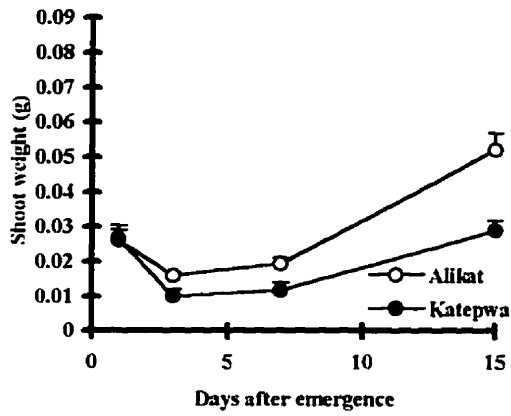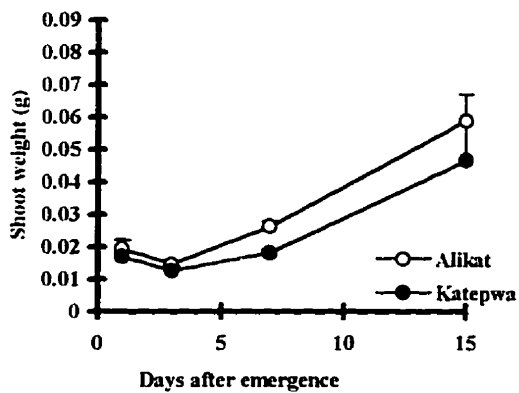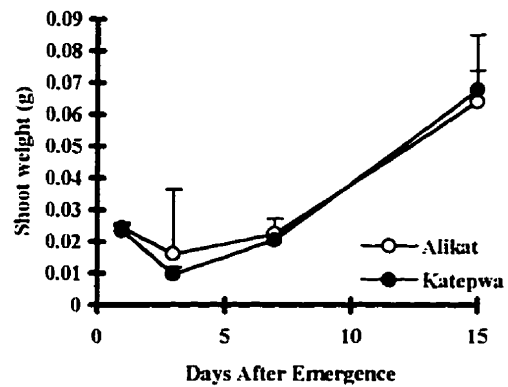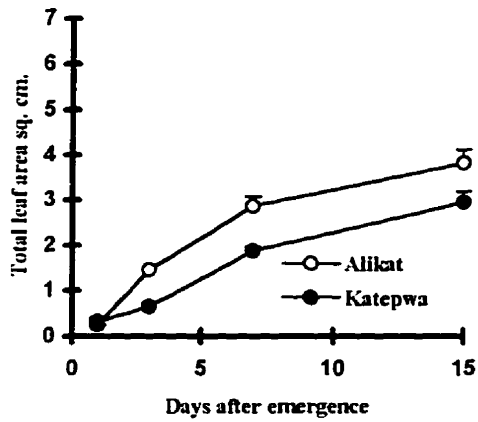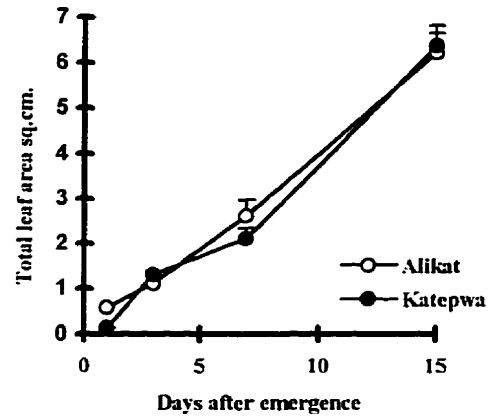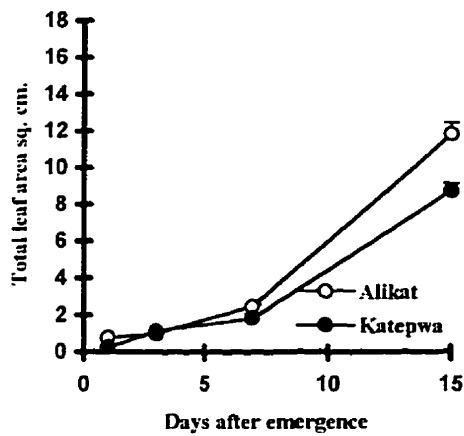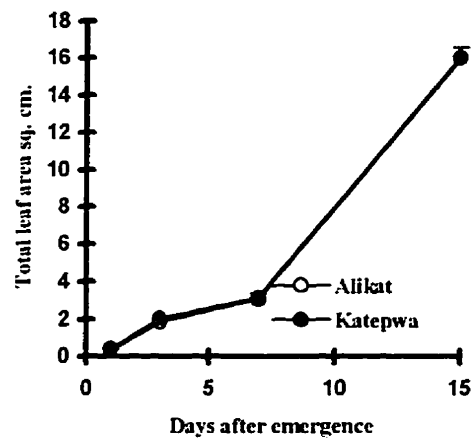
Fig. 4.2. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0

Fig. 4.3a. Root weight at pH 4.4
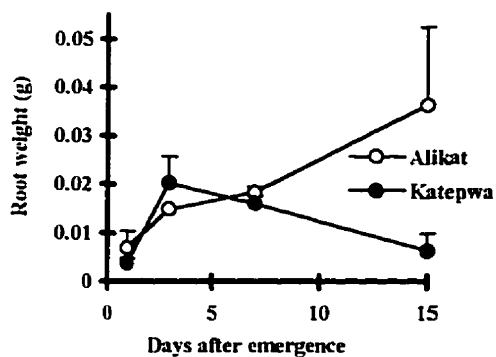
Fig. 4.3b. Root weight at pH 4.9
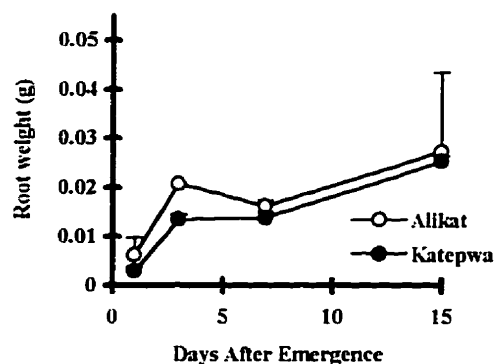
Fig. 4.3c. Root weight at pH 5.5
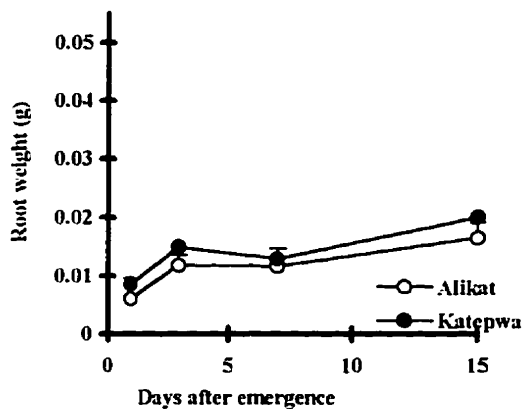
Fig. 4.3d. Root weight at pH 7.0
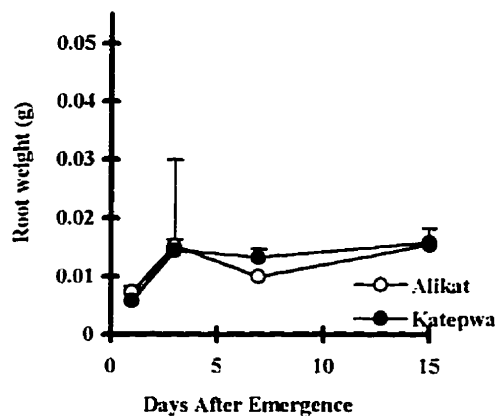
Fig. 4.3. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0

Fig. 4.4a. Shoot weight at pH 4.4

Fig. 4.4b. Shoot weight at pH 4.9
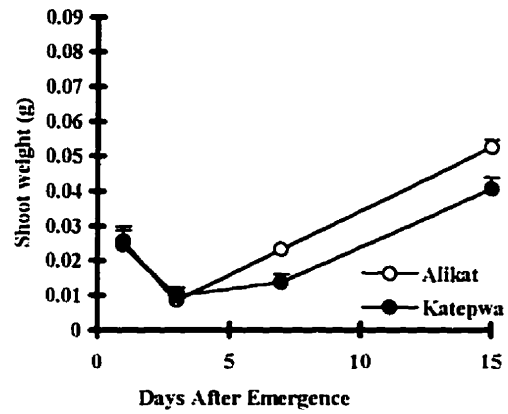
Fig. 4.4c. Shoot weight at pH 5.5

Fig. 4.4d. Shoot weight at pH 7.0

Fig. 4.4. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0

Fig. 4.5a. Total leaf area at pH 4.4

Fig. 4.5b. Total leaf area at pH 4.9

Fig. 4.5c. Total leaf area at pH 5.5
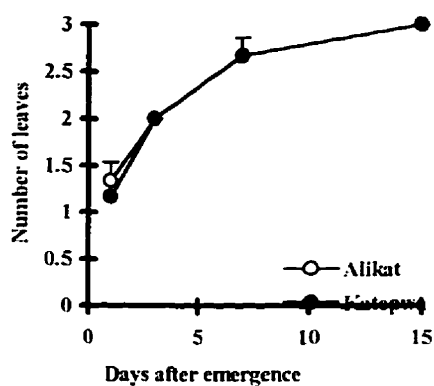
Fig. 4.5d. Total leaf area at pH 7.0

Fig. 4.5. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0

Fig. 4.6a. Total number of leaves at pH 4.4
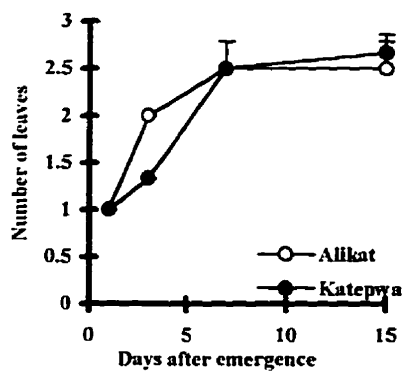
Fig. 4.6b. Total number of leaves at pH 4.9
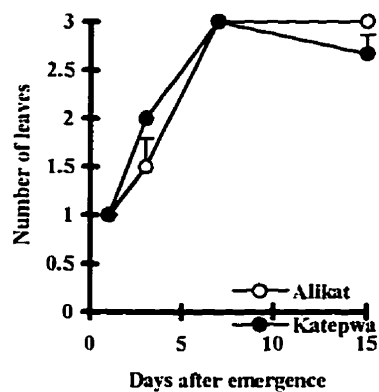
Fig. 4.6c. Total number of leaves at pH 5.5
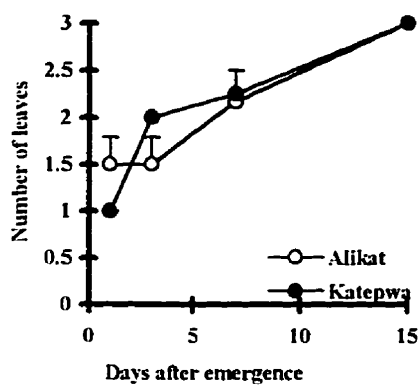
Fig. 4.6d. Total number of leaves at pH 7.0

Fig. 4.6. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0

Fig. 4.7a. Length of the first leaf at pH 4.4
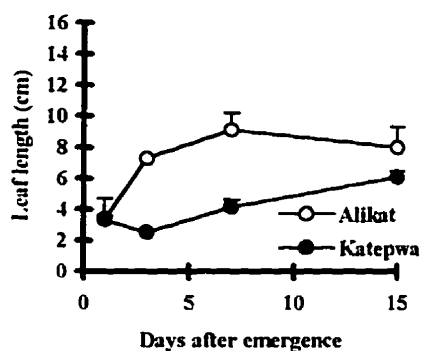
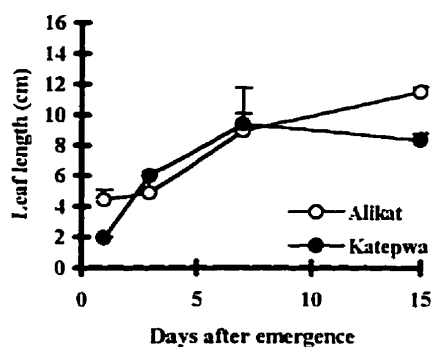Fig. 4.7b. Length of the first leaf at pH 4.9

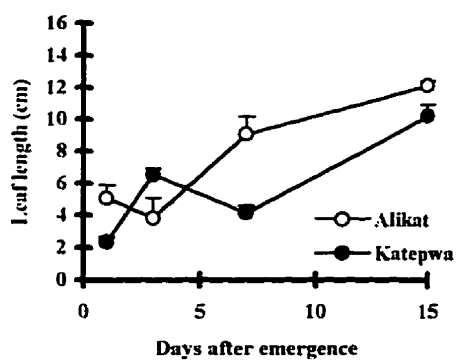Fig. 4.7c. Length of the first leaf at pH 5.5

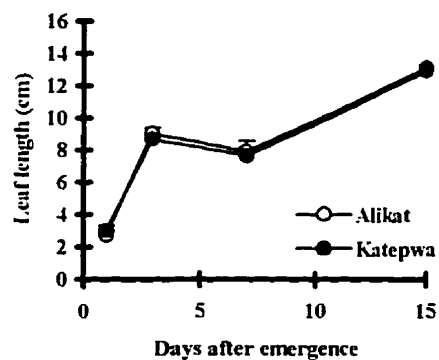Fig. 4.7d. Length of the first leaf at pH 7.0

Fig. 4.7. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0
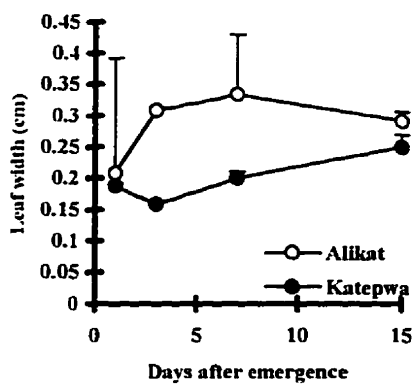
Fig. 4.8a. Leaf width of the first leaf at pH 4.4

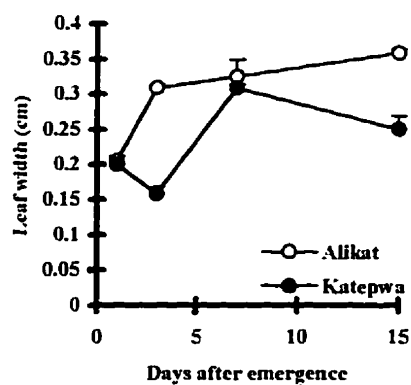

Fig. 4.8b. Leaf width of the first leaf at pH 4.9

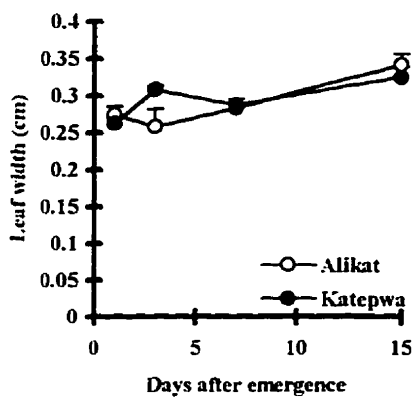Fig. 4.8c. Leaf width of the frst leaf at pH 5.5

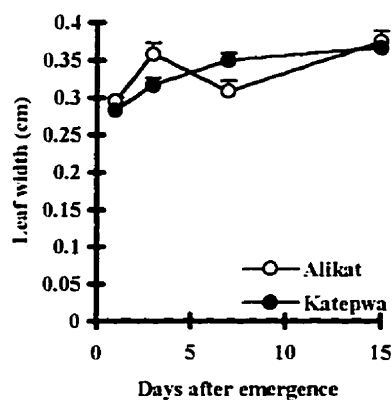Fig. 4.8d. Leaf width of the first leaf at pH 7.0

Fig. 4.8. Developmental characteristics of Alikat (aluminum tolerant) and Katepwa (sensitive) cultivars grown in the greenhouse in an acid humic gleysol limed to different soil pH levels (a) pH 4.4; (b) pH 4.9; (c) pH 5.5; (d) pH 7.0

4.5 References

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Chapter V

Summary

Differential response to aluminum stress of Alikat/Katepwa near-isogenic pair for has been well characterized. Studies in nutrient media have shown that the Maringa source of aluminum tolerance introduced into Katepwa performs almost as well as Maringa at a high level of toxicity of 600 µM of Al in solution. Further field studies have demonstrated that Alikat (Al-tolerant) yields the same as does Katepwa (Al-sensitive) in neutral soils. Molecular studies of stress induced polypeptide production in this pair of genotypes have been done which could lead to an explanation of the aluminum tolerance mechanism offered by the particular gene in question. However, the range of soil pH and the associated aluminum toxicity levels under which the tolerance gene provides an advantage to a wheat plant growing in acid soil has not been previously studied.

In Al-toxic acidic humic gleysol from Silver Valley, the root growth of wheat plants of Alikat and Katepwa genotypes were differentially affected at low pH levels between 4.4 to 5.5. This pH range of effectiveness reconfirmed reports from previous findings. However, the root growth above this pH (5.7-7.0) was not as expected as Alikat continued to grow better roots than Katepwa in this pH range where the Al was not toxic. A possible explanation could be the variability occurring in other factors such as Mg, Ca, or P including deficiencies induced by various pH levels. It was clear that Alikat produced more than twice the amount of roots than Katepwa and this would place Alikat at an advantage in terms of nutrient, water uptake, plant establishment and top growth. Shoot growth was similarly affected but the response was not as large as seen in the roots. Shoot growth of Alikat in the pH range 4.4 to 5.7 was far better than Katepwa but there was large variation for Alikat which made the trend less clear. It is already known that there is a lower responsiveness of shoot growth to acid soil stress, compared to root growth. Total biomass of Alikat was about four times higher than Katepwa at the lowest pH. The rise of total biomass of Alikat plants with increasing doses of lime application compared with that of Katepwa showed that Katepwa was more responsive to lime amendments as would be expected. The experimental protocol was important in these experiments. Plants grown to maturity did not show as clear a level of damage due to toxicity because of changing conditions of stress with time. When plants are grown for a short period of time (i. e., flowering stage) differential effects were much more obvious on plant traits compared to effects assessed at

maturity. This is consistent with a physiological model which attributes primary stress responses of aluminum where plants grow to maturity to negative effects in early seedling stages.

Long term aluminum toxicity studies showed that the aluminum tolerance gene also produced a difference in seed yield, total biomass, harvest index of the tolerant and sensitive plants in the soil pH range from 4.4 to 5.7. Despite yield being a multigenic trait, the aluminum tolerance source seemed to present an advantage to plants which were more tolerant to the stress. Results also showed that with less lime application the maximum yield potential could be reached by the tolerant plants. This difference in yield performance could either be caused by better root growth which helped in nutrient and water uptake or could be because of better sunlight-assimilating capability or by both. For this reason the rate of development and more specifically, leaf appearance, leaf development, and total leaf area development were studied.

Leaf area development of Alikat under very acid soil pH was more rapid than that of Katepwa, conferring a distinct advantage to these plants, and this was also associated with shoot and root weight increase. The pH range of 4.4 to 5.5 seemed to be crucial for the sensitive Katepwa plants and at pH 7.0 both Alikat and Katepwa grew at similar rates. Leaf lengths of the first leaf in Alikat plants were larger than in Katepwa plants. This component contributes to greater photosynthetic area seen in the tolerant Alikat plants at very acid soil pH range (4.4-5.5). The Katepwa plants were not only challenged by poor root growth they were further slowed down in their assimilation potential by lower total leaf area. This is a new finding in the area of aluminum toxicity and its relation to yield since prior studies concentrated only on describing root effects on yield potential. So far, poor root growth had been stated to be the main reason for lowering of yields but with these results it appears that leaf area development may be affected by aluminum toxicity stress. Use of the near-isogenic lines in this study helped considerably in indicating the associated effects of aluminum on the physiology and development of wheat grain yield under aluminum stress.

The most important contributions of this study were twofold. Firstly, it was determined that aluminum tolerance from Maringa is effective in an acid soil system in a manner consistent with prior findings in hydroponic studies and the gene is effective in the pH range below 5.5-5.7. Secondly, it was also determined that in addition to reconfirmation of negative effects on root development in intolerant genotypes growing at low soil pH, it was also confirmed that leaf area development is also greatly affected in a negative manner. These

effects during the early seedling growth can have bearing on yield. Although the near-isogenics are theoretically similar up to 87.5% in their genetic makeup, these studies have offered valuable insight into the role of the genetic source of aluminum tolerance in root, shoot, leaf growth and yield in response to lime application. The economic value of this gene could be quantified by conducting trials at various acid soil sites. A comparison of the cost of liming in such sites with growing a tolerant variety may clarify the advantage of breeding an aluminum tolerant variety.